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# The Impact of a Naturalistic Stressor on Spontaneous Alternation Behavior: A New Animal Model of OCD

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Abstract

Over the past few decades, various animal models of obsessive compulsive disorder (OCD) have been developed. Similarly, various stressors have been used throughout animal research. The Spontaneous Alternation Behavioral (SAB) model is a well-established model of OCD while 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) has recently become a popular naturalistic stressor. This study linked the two together, thus modeling the effect of stress on OCD behaviors. After living in an enriched or standard environment for 3 weeks, male Sprague-Dawley rats were exposed to either TMT or no odor, and then were examined in the SAB task. Unlike what was hypothesized, the enriched environment proved not to be obviously protective towards future stress in terms of SAB behavior. However, rats housed in enriched environments proved to be more decisive, which could be reflective of the protective nature of their enriched housing. Additionally, an interaction effect of housing and odor in terms of distance traveled during odor exposure, paired with the rats' location in regard to the odor source, lead the researchers to believe that the enriched housing was protective for rats faced with stress. TMT proved to be effectively aversive to the rats yet, unlike what was hypothesized, TMT was not shown to be stress-inducing, at least not in a way that increased OCD-like behavior as modeled by the SAB model. The effects of stress on OCD is challenging to model and further research in this field, using a variety of models, will need to be explored.

*Keywords*: OCD, stress, SAB, TMT, enriched environment

Table of Contents

The Impact of a Naturalistic Stressor on Spontaneous Alternation Behavior:

A New Animal Model of OCD

Over the past few decades, various animal models of obsessive compulsive disorder (OCD) have been developed. Similarly, various stressors have been used throughout animal research. The Spontaneous Alteration Behavioral (SAB) model is a well-established model of OCD while 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) has recently become a popular naturalistic stressor. Despite their individual popularity, previous studies linking the two have not been conducted. This study linked the two together, thus modeling the effect of stress on OCD behaviors. The protective measures of an enriched environment are also well established so this was included in the present study in the context that rats living in standard conditions would be more susceptible to future stress than rats living in an enriched environment.

Stress affects everyone to some degree and can be especially detrimental to those already suffering with an anxiety disorder. The impact of stress on anxiety disorders has been widely accepted yet not extensively documented. A study conducted by Lin et al. (2007) monitored stress levels in children and adolescents and the associated fluctuations in their anxiety disorders. Not only did OCD patients experience significantly more psychosocial stress than controls, but results also indicated that increased stress was a strong predictor of increased OCD symptoms.

**Obsessive-Compulsive Disorder**

Obsessive-compulsive disorder (OCD) is a debilitating anxiety disorder comprised of two distinct phenomena, obsessions and compulsions. Obsessions are

recurrent, obtrusive thoughts and urges whereas the compulsions are repetitive behaviors performed as a means of coping with the obsessions. In humans, OCD behaviors often include repetitive checking, washing, hoarding, or ordering of objects. Additional stressors can aggravate symptoms, making life difficult for individuals with OCD.

OCD affects millions of people around the world and is characterized by repetitive behaviors reflecting recurrent obsessive thoughts. OCD is one of the most common mental disorders, affecting about 2% of people across the globe. At diagnosis, patients experience obsessions or compulsions (e.g., doubting, checking, washing) that are time consuming (more than one hour per day) or cause distress and impair normal living (Bartz & Hollander, 2006). People with OCD usually realize their behavior and thoughts are abnormal, but yet still feel compelled to act out their OCD behaviors. OCD behaviors vary greatly across people, and examples range from minor sorting or checking habits to extreme repetitive behaviors so harsh they can cause self-injury.

OCD has a high heritability and most people present symptoms before the age of 18. Although the disorder has the same prevalence in men and women, men with OCD commonly have an earlier onset than do women. Concrete causes of OCD have not been identified but it is widely understood that psychological, biological, and environmental factors are important. Recent studies have found inheritable differences in the genetic coding for the protein expression of serotonin receptors, confirming the biological link of OCD prevalence (Goddard, Shekhar, Whiteman, Christopher, & McDougle, 2008). From an evolutionary psychology viewpoint, OCD behaviors such as excessive washing and hoarding were once advantageous, and a heritable genetic mutation in the serotonin transporter gene has been found in many people with OCD. Although it is not clear

whether this is a cause or effect of OCD, people with OCD have been found to have serotonin abnormalities or under-stimulated serotonin receptors. OCD is often co-morbid with depression as well as a number of other conditions, including other anxiety disorders and Tourette's syndrome. These co-morbidities reflect the anxiety and repetitive behavior associated with OCD as well as a link to the serotonin system, as all can often be treated with SRIs. Environmental factors, particularly stresses in early childhood, have also been linked to increased prevalence and severity of OCD.

Even though the precise physiological and psychological causes of OCD are not fully established, it has been shown that there are a number of neural systems involved. Three major neurotransmitter systems have been hypothesized as being critical to the development of OCD: the serotonergic, the dopaminergic, and the glutamatergic systems (Albelda & Joel, 2011). While the serotonergic system has been studied the most, the important roles of dopamine and glutamate have recently been recognized. Dopamine reuptake blocking medications have been used to successfully treat people with OCD, alluding to the strong likelihood of the dopaminergic neurotransmitter system being involved in the neuropathology of OCD. The importance of the glutamatergic system is reflected in observed differing levels of glutamatergic metabolites that correspond to OCD symptom severity (Albelda & Joel, 2011). All neurotransmitter pathways are intertwined in the behaviors they regulate, for that reason, it is difficult to isolate and identify the specific effects of each individual system.

Divergence from the usual serotonergic (5-HT) system in OCD patients has been recognized primarily on the basis of the effectiveness and widespread use of serotonin re-uptake inhibitors (SRIs) and selective serotonin re-uptake inhibitors (SSRIs) in

alleviating obsessional and compulsive behaviors in humans. From the widespread success of SSRIs as a drug treatment for OCD, serotonin clearly plays an important role in the behaviors characterizing this disorder. From this, the serotonergic system has become the primary focus of the pathophysiology of OCD and other anxiety disorders. Recent imaging studies, which identify specific areas of activation in the brain, support a strong link between serotonin receptors and OCD. Specifically, reduced cortical serotonin levels and increased serotonin binding were identified in the caudate nucleus of human OCD subjects, compared with healthy controls in a study using positron emission tomography (PET) scans (Simpson et al., 2011).

Unlike other disorders, where abnormalities can be pinpointed to specific areas of the brain, OCD is very complex and the neural circuitry behind the disorder is not well understood. However, there is a fairly universally believed cortico-basal ganglia circuit dysfunction, often referred to as the "OCD circuit" or "OCD loop," which indicates the involvement of many different structural elements that impact the manifestations of OCD (Graybiel & Rauch, 2000).

Areas in the "OCD loop" include the orbitofrontal cortex (OFC), the anterior cingulate/caudal medial prefrontal cortex (PFC), and the caudate nucleus (part of the striatum area of the basal ganglia). Activation of these areas has been shown to be higher in those with OCD compared to healthy controls. These areas are activated even further when OCD behaviors are exacerbated, while successful treatment of OCD diminishes previously high levels of activation (Graybiel & Rauch, 2000). Additional neuroimaging studies of OCD patients have also implicated abnormal or excessive metabolic activity in the OFC, the PFC and areas within the basal ganglia (Albelda & Joel, 2011). Many

different studies using various detection methods have concluded that these same brain areas play a key role in OCD, further supporting the "OCD loop" theory.

The caudate nucleus, putamen, and pallidum are components of the basal ganglia, which plays an important role in movement control. The likelihood of basal ganglia involvement in OCD is high, reflective of data from abnormal metabolic activity in neuroimaging and lesion studies of areas within the basal ganglia leading to induced OCD-like behavior. Obsessions and compulsions can be viewed as the loss of normal control over thoughts and movements that can be traced back to abnormalities in the basal ganglia's normal regulatory role between the neocortex (higher thinking and general motor commands) and the limbic system (emotions and emotionally driven behaviors).

The information provided by brain imaging studies has led to treatment approaches in which components of the OCD loop are manipulated in order to alleviate symptoms. Lesion and deep brain stimulation (DBS) studies have been extremely useful both in identifying the brain areas involved with OCD as well as in providing potential therapeutic means of treating the disorder. DBS is a developing field and OCD has been at the forefront of disorders being treated by targeting specific brain areas involved (Greenberg, Askland, & Carpenter, 2008). DBS is a powerful tool in understanding and treating OCD because of the precise focal points of this applied technology as well as the reversible nature of the therapeutic interventions. To some degree, DBS can be viewed as temporary lesioning.

Lesioning studies are much more intrusive and are permanent, so animal models are needed to allow researchers to further assess the role of various brain areas in

behaviors associated with OCD. A lesion study by Schilman, Klavir, Winter, Sohr, and Joel (2010) determined that lesions of the OFC increased compulsivity in rats, confirming the likely role of the OFC in animal OCD behavioral regulation. Additionally, it can be extrapolated from animal studies that the compulsive behaviors of OCD patients are manifestations of disregulation of the striatal serotonergic system caused by initial pathology in the OFC. A similar study by Joel, Doljansky, Roz, and Rehavi (2005) also demonstrated that OFC lesion-induced compulsivity (lever pressing) was prevented by administration of an SRI. This evidence provides predictive validity to the now well-established link between dysfunction of the OFC and OCD. Additionally, focal lesions in the striatum as well its target structures in the basal ganglia produce OCD-like behavior (Laplane et al., 1989).

There are numerous studies that successfully link various brain areas to OCD-like behavior based on changes in behavior reflecting physical changes within the brain. Such an array of manipulations, often intrusive and complex, would not be possible in human subjects, thus leading to the need for animal studies. Animal studies have been an instrumental tool in understanding OCD and providing scientific support for the continuing growth of knowledge about the neurobiology of OCD.

**Mechanisms of Stress and OCD Associated Neurotransmitters**

Stress is a very interesting and complicated response to stimuli and has been extensively studied. To varying degrees, there is stress in all of our lives. Stress can be adaptive, keeping us away from dangers and focusing our minds on the most important issues at hand. However, in today's world, stressors often arise from non-life threatening

situations and create significant physical and mental/emotional strain and distress. OCD is primarily an anxiety disorder, and stress levels in individuals with OCD are already above normal levels; added stress only exacerbates OCD behaviors (Lin et al., 2007). Amplified OCD behavior has been linked to increased cortisol levels, demonstrating that the level of stress and severity of OCD symptoms are closely linked (Lord, Hall, Soares, & Steiner, 2011).

Acute stress increases OCD behaviors in humans as well as the OCD-like behavior of laboratory rats. An example of an acute human stressor is the Trier Social Stress Test (TSST) in which the participant must orally present in the form of a job interview before a panel of strangers. The TSST has been widely used as an effective stressor with consistent activation of the hypothalamus-pituitary-adrenal axis (HPAA) and the autonomic nervous system (ANS), noted by increased heart rate and stress-hormone levels (Hellhammer & Schubert, 2012). In comparison to controls, participants in the midst of, as well as after having undergone TSST, are found to have higher levels of stress, anxiety, and emotional insecurity, which all can be seen as parallel feelings to those of mammals exposed to stressors in their natural habitats.

It is challenging to identify a single mechanism by which stress exerts an impact on OCD because many neurotransmitters are thought to be involved in OCD, and stress affects multiple neurotransmitter systems. For example, dopaminergic neurons respond to stress by increasing their rate of firing, possibly causing physical changes in neuronal pathways that may lead to maladaptive behaviors. Dopamine is largely known for being involved in the brain functions of reward, pleasure, fine motor function, compulsion, and perseveration. OCD behaviors may be caused by a combination of hindered PFC

processing and habit-forming behaviors being prompted by the increased dopamine levels in the basal ganglia (Arnsten, 2009). Increases in basal ganglia size have also been linked to OCD (Giedd, Rapoport, Garvey, Perlmutter, & Swedo, 2000). This outcome makes sense if we realize that other subcortical areas, such as the basal ganglia, likely take over some functions usually controlled by the PFC. In its highly stressed state, the PFC is unable to cope and the basal ganglia and other areas try but are unable to fully filter the neural signals as the PFC normally does. Increased dopamine levels in the basal ganglia prompt habit-forming behaviors. Thus, increased stress can lead to worsening of maladaptive behaviors by both the (hindered) PFC processing and the increased dopamine levels in the basal ganglia.

When faced with environmental stressor, serotonergic neurons also play an important role in an animal's reaction. As a major modulatory neurotransmitter, serotonin (5-hydroxytryptamine, 5-HT) has diverse behavioral effects system-wide in animals. As applied to the brain, stress, and OCD, 5-HT plays an important role in mood regulation, sensorimotor reactivity, memory processing, and learning – all of which can be affected by stress. The release of 5-HT is important in normal nerve signaling and low levels of this neurotransmitter have been linked to a number of psychiatric disorders, including impulse-related disorders as well as depression and anxiety disorders, including OCD. The 5-HT system is a complicated and intricate one, with serotonergic neurons being highly bifurcated and thus ideal for innervating a vast distribution of CNS areas simultaneously, from the spinal cord to the cortex (Lucki, 1998).

Given that in the present study OCD-like behaviors were induced with a serotonin agonist, the mechanisms of OCD involving serotonin are important to address, even if not

fully understood. Lucki (1998) discusses the limitation and inconsistencies for various general theories of 5-HT and behaviors. One example of variation in findings, as explained in Lucki's (1998) article, was that forced swimming of rats (a type of stressor) produced both increased, reduced, or no change in levels of 5-HT, depending on which study was evaluated. More specifically relevant to OCD, Goddard et al. (2008) reflect on conflicting evidence of the density of serotonin in brain areas of people with OCD; their article proved that separate attempts to map out the brain areas associated with abnormal levels of serotonin did not have the same findings. From the endless amounts of conflicting data, it seems nearly impossible to determine a comprehensive and uniform connection between behavioral state and patterns of 5-HT release.

Despite vast differences, abnormal reductions of 5-HT in the thalamus/hypothalamus, midbrain, and brainstem in OCD patients were supported by a number of studies using positron emission computed tomography imaging assessments cited by Goddard et al. (2008). When put into the context of OCD, the behaviors of OCD reflect the likely 5-HT deficiency in associated brain areas. The thalamus has intricate regulatory connections with other brain areas so abnormal 5-HT levels could be representative of the widespread neural associations of OCD. The brainstem plays an important role for the motor and sensory systems; if 5-HT levels were to decrease as in the case with OCD, motor and sensory control could also decrease, which could be linked the compulsions associated with OCD.

The impact of stress is also serotonergic. Relating back to the article by Goddard et al. (2008), the hypothalamus in particular is very responsive to stress, adding to the behavioral decline of OCD patients exposed to stress. A study conducted by Jasinska,

Lowry, and Burmeister (2012) suggests that, based on both animal and human studies, genetic variation in the 5-HT system affects the stress response, which is regulated by the amygdala, ventromedial prefrontal cortex (VMPFC), and the dorsal raphe nucleus (DR). Among other things, the VMPFC is implicated in the processing of decision making, which is reflected in the OCD behavior of uncontrollable actions, where decision making is possibly lacking or overrun by impulses elsewhere in the brain. The DR is arguably the most important brain area in terms of serotonin as it is the largest serotonergic nucleus and innervates much of the forebrain. In addition to being important for sending messages to the cortex, areas of the raphe nuclei are associated with vigilance, a quality that when it becomes abnormal, can be linked to the compulsive checking seen in people with OCD. The brain areas and neurotransmitters linked to OCD are not exhaustive, as much research still needs to be conducted. However, even from these few examples, the importance of neurotransmitters and how they relate to OCD and are affected by stress can be appreciated.

**Examples of Types of Stressors**

One of the biggest challenges in understanding the role of stress is finding comparable stressors for animals that parallel the stress experience in humans. For humans in today's world, the most common stressors arise from social interactions, with truly life-threatening situations infrequently occurring. In animals however, the most common natural stressors (that humans can detect) are of a life-threatening nature. Even initially, the stressors across species are not consistent, so further analysis and treatment of excess stress may reveal that the responding brain circuits are not necessarily identical.

Predator odor stress might be an applicable stimulus because, like the social stressors in humans, it is a naturalistic stressor that causes no direct physical trauma. There are a number of commonly used acute stressors of rats in laboratory settings along with a plethora of ways to test for stress. Mercier, Canini, Buguet, Cespuglio, Martin, and Bourdon (2003) determined that there are differences in type and severity of behavior between rats exposed to different types of stressors, namely restraint, forced swim, and inescapable foot shock. In that article the authors explain the inability of researchers to scientifically estimate the perceived stress of the animal even while testing the classical physiological variables of body temperature and blood cortisol levels. Another experiment (Gutiérrez-García et al., 2006) tested the acute physiological stress of rats by having them visually and audibly witness another rat experiencing foot shock. The determination of stress level was measured by the duration of burying behavior exhibited by the rat as well as the amount of alarm pheromone detected in the rat's urine. There are also many types of chronic stressors used in rat research, which have been shown to reduce neuronal dendrite length, branching, and spine density, along with a variety of behavioral effects. Common methods of chronic stress include social isolation or differing types of housing environments.

**Predator Odor as Acute Stressor**

In the present study, exposure to predator odor was used as the acute stressor. The physical modes of stress described previously often involve pain pathways rather than just the stress/fear/anxiety pathways. Additionally, many methods (like inescapable foot shock, etc.) place the rat in stressful scenarios unlike anything that would occur in

the rat's natural environment. For this reason, the use of predator odor is arguably a realistic and appropriate stressor. As previously explained, using a non-physical stressor fits the design of this project well; human stressors that trigger OCD behaviors are normally non-physical in nature. Also, because the control subjects of the study were not to be exposed to the stressor at all, it was beneficial to avoid having to physically disturb the rats or significantly alter their physical settings in order to limit potential sources of error in our data. In addition, predator odors elicit specific defensive and risk assessment behaviors in rats that are generally distinct from behaviors elicited by noxious or novel odors (Dielenberg & McGregor, 2001).

**TMT**

The chemical compound 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) is a synthetic substance isolated from fox feces. The fox is a natural predator of the rat; for that reason naive rats are innately stressed when exposed to foxes. Although not found in other predators of the rat, it should be noted that TMT is not unique to the fox; it has also been isolated from cooked beef and wheat flour (Fendt, Endres, Lowry, Apfelbach, & McGregor, 2005).

TMT has played an important role in lesion studies illuminating the neuronal pathways and brain areas involved in fear and anxiety. When combined with lesion studies, the effect of TMT has contributed to a further understanding of the brain areas associated with anxiety disorders. For example, the effect of TMT on freezing behavior has led to the hypothesis that the bed nucleus of the stria terminalis (BNST) is important in anxiety whereas the amygdala is more important in fear (Fendt et al., 2005).

**TMT Justification**

TMT has become increasingly popular over the past few decades as an acute stressor of rodents in experiments surrounding fear and antipredator behaviors. A study by Fendt et al. (2005) outlines that both laboratory and wild-caught rats exposed to TMT exhibit behaviors and stress-hormone levels typical of stressful situations. Avoidance of the odor source, decreased grooming behavior, increased freezing, and increased defecation have been noted across many studies as the tell-tale behavioral signs of stress. These behaviors have been reported both in rats exposed to TMT as well as other sources of predator odors (Endres, Apfelbach, & Fendt, 2005). Fendt et al. (2005) however, describe how freezing, thought to be one of the most prominent behavioral signs of fear in rats, is not a reliable behavioral response of rats exposed to TMT. This variation in experimental conclusions creates uncertainty surrounding the validity of TMT use.

TMT has been criticized for being more than just a predator stimulus. TMT is an offensive, even noxious, stimulus that may be more potent than odors that occur in nature. Studies comparing forms of predator odor report differences in behavior, suggesting TMT is more noxious than predatory when compared with cat odor. Specifically, TMT has not been shown to elicit risk assessment (head out positioning from a hiding place) or fear conditioning behaviors that are considered characteristic defense responses in rats. TMT also has been found to elicit extremely defensive behavior that may reflect more than just predation responses (Wallace & Rosen, 2000).

An experiment conducted by Staples, McGregor, Apfelbach, and Hunt (2008) compared the behavioral and neural activation of rats elicited by cat odor, TMT, and

formaldehyde (an acrid, noxious, but non-predatory odor). Findings showed significant behavioral and neural differences between animals exposed to cat odor and TMT. Specifically, only rats exposed to cat odor exhibited escape attempts and complete inhibition of adaptive behaviors such as grooming. In the brain, cat odor produced an activation pattern associated with defensive behavior whereas TMT produced activation primarily typical of only a volatile and noxious compound. Cat odor is usually undetectable by humans whereas TMT is an easily detectable, malodorous substance. McGregor, Schrama, Ambermoon, and Dielenberg (2002) sought to determine whether it was the offensive nature of TMT that resulted in differing rat behavior. They did an experiment using a putrid fish substance, TEA, and determined that rat behavior following exposure to TMT is based on the noxious quality of the substance rather than the predation quality.

TMT has been shown to activate the piriform cortex, bulbus olfactorius, anterior cingulate cortex, and the horizontal limb of the diagonal band of Broca (Staples et al., 2008). These areas also are activated by cat odor and formaldehyde, suggesting that beyond acute stress they play a more general role in olfactory responsiveness. Specific areas that are activated by cat odor and TMT were the ventral orbital cortex, piriform cortex, and the anterior cortical amygdala. However, there were dozens of additional brain areas activated by cat odor but not TMT, namely the dorsal premammillary nucleus (PMd), dorsomedial part of the ventromedial hypo- thalamus (VMHdm), and anterior hypothalamus (AHN). These specific areas are thought to comprise the neural components of the rat's defense system. They concluded that the failure of TMT to activate the defense system means that TMT likely does not elicit a full predatory threat.

Although several studies show that TMT is not comparable to cat odor, many also have shown TMT to be a suitable stressor. Unlike conclusions of other researchers, Fendt and Endres (2008) do not feel there is enough experimental support of direct brain activity comparisons between TMT and cat odor. However, they find TMT to be a suitable stressor based on similarities in behavioral and neuronal responses to both TMT and cat odor. They argue that these responses cannot simply be dismissed as in other studies' simplistic findings that TMT is merely repugnant. Multiple studies relate results from TMT-induced brain activation patterns as reflective of the presence of fear and associated defensive patterns (Fendt, Endres, & Apfelbach, 2003). Fendt et al. (2005) explicitly evaluate the validity of TMT as a stressor. Face validity is shown through the fearful behaviors of rats exposed to TMT being similar to those expressed by fearful humans. Construct validity is supported through a number of studies citing similar brain areas (specifically the limbic system-including the amygdala, hypothalamus, BNST, etc.) between stressed/fearful humans and rats. Predictive validity is a bit more challenging to claim because there have been limited studies and the ones that have been discussed do not support TMT-induced fear as being treatable by the same anxiolytic agents that treat humans or rats exposed to other stressors, including cat odor.

Given such varied conclusions on the effectiveness of TMT, there is no consensus on the best predator odor stressor. Some researchers conclude that TMT is an effective stressor, but believe it to follow a different neural pathway than other stressors; even the response can differ between rat species (Morrow, Redmond, Roth, & Elsworth, 2000; Rosen, West, & Donley, 2006). Assuming the synthesis of TMT produces a substance comparable in nature to that found in actual fox feces, discrepancies in the types of stress

induced could also be responsible for variation in the resulting behavior and neuronal activity. Cat odor, which comes from the skin and fur of a cat, signifies an immediate presence of the cat and thus an immediate life-threat. The odor of fox feces represents a less-immediate threat because at the time of detection, the predator who defecated is not necessarily still in the immediate area. In fact, the species of red fox, the feces of which TMT was created to mimic, are known to strategically defecate outside of their hunting areas. This action is presumably to create the element of surprise when stalking their rodent prey who otherwise would be fearful and thus more careful not to be caught. Cat odor is a time sensitive stressor whereas TMT mimics a long-term stressor, so different types of information are likely neurally transmitted in different capacities. Thus, it is not surprising that behavioral manifestations of the stressors may differ.

For the present study, cat odor was considered but disregarded in favor of TMT. The use of cat odor also involves confounding variables, especially with consistency and variation. Since its active components are unknown and unquantifiable, it would not necessarily be constant across trials. There are multiple methods of collecting and accessing cat odor (including cloth collars worn, cloth or bedding in contact, urine/feces from the cat, etc.) that allow for variation in odor strength in addition to the innate distinctiveness of each cat donor. Even if the initial source of cat odor remains constant, variation continues based on how much time has elapsed since the sample was collected. However, TMT can be purchased as a pure odor, which eliminates all possible variation within and between studies.

Given the drawbacks of using cat odor, TMT was concluded to be the best way to ensure consistent and predictable predator odor. Since the present study compares the

behavioral results of stress versus no stress, rather than variation based on types of stressors, the consistency and reliable accessibility of TMT was valued above the authenticity of natural cat odor. The Staples et al. (2008) study also noted that Sprague-Dawley rats, like those used in the present study, have been shown to have a greater sensitivity to TMT than have other strains of rats, such as the Wistar rats used in their study. TMT was the chosen acute stressor for the present study and its use was combined with environmental manipulations.

**Enriched Environment Protective against Subsequent Stress**

Literature on the effects of enriched environments versus standard environments can be traced back to the 1800s. Experiments conducted over a century ago allude to the benefits of an enriched environment, a hypothesis that is still strongly supported today. A recent study on quails shows that an enriched environment improves an animal's immune response and counteracts the physiological effects of stress exposure; in essence showing chronic stress to be the opposite of environmental enrichment (Nazar & Marin, 2011).

Environmental factors play an important role in the physical and mental well-being of all animals, including humans. Factors such as infections in infancy or pregnancy, early parental loss, and child abuse have been shown to have negative effects in animal studies, whereas an enriched environment has been shown to have a positive effect against acquired pathogenesis (Takuma, Ago, & Matsuda, 2011). In the present study, we hoped to duplicate the preventative effect of an enriched environment, as validated in that study.

Rats are very social and inquisitive animals who thrive in enriched environments.

Rats living in enriched environments are much less susceptible to negative influences and show an increase in spatial memory ability compared to rats living in standard environments (Wright & Conrad, 2008). In the present study, states of relative chronic stress were provided for half of the rats in the study by means of minimal home-cage environments (with no shelter or wheel), whereas the other the rats lived in enriched home-cage environments (with a shelter and wheel) and were given "rat recess" three times per week. While other studies switched housing pairs daily to achieve an enriched environment, paired rats remained together in the present study but during "rat recess" were allowed to play with three other enriched environment rats in a large tub with many novel objects.

**Animal Models of OCD**

Animal models have been used for decades to test various aspects of human conditions, afflictions, and potential treatments, but it is impossible for one animal model to portray all aspects of a targeted human condition, including OCD. Different animal models have validity for different aspects of the disorder being studied. One criterion for judging the quality of a model of mental illness is its "face validity." Face validity means that it looks like what it is; at face value the animal appears to have the same human affliction. Increased checking behaviors (as is shown via the SAB model) is an example of supportive face validity in a rat with OCD as such behaviors are prevalent in humans with OCD. Face validity is especially important in animal models of OCD because this type of validity has been widely supported (by parallel human OCD behaviors), whereas other forms of validity (that often rely on brain areas) have proven more challenging to

support. Joel (2006) compares some of the most common types of animal models used in OCD and reflects on their validity. The three main animal model classes for OCD are genetic, pharmacological, and behavioral. All specific experimental procedures falling under these categories are supported by various degrees of validity.

Concerning OCD, the predictive validity of animal models is especially tricky because of the widespread use of SRIs in treatment of several psychiatric disorders. For that reason, it is challenging to identify an animal model that specifically captures OCD because the SRI that works to treat it also can successfully treat depression, anxiety, and a variety of other psychiatric disorders. Additionally, the extended time-frame within which SRIs show their effectiveness, as well as the anticipated percentage of patients who are simply unaffected by SRIs, makes determining the effects of OCD treatment challenging in animal studies. Across all types of OCD animal models, a common theme is the lack of extensive research, thus preventing the compilation of conclusive summaries of the effectiveness of each model.

Genetic models include four mice models of OCD based not on known genetic OCD-relevant mutations in humans, but instead on the behavior of the mice. These mice have specific, known genetic changes that in turn cause them to behave in an OCD-like manner (e.g., excessive grooming, patterned chewing, etc.). Although brain areas shown to be most affected by these genetic changes align with those previously found to be related to OCD, the predictive validity of the genetic models is possible, but currently not verified (Joel, 2006).

Behavioral models are not popular in current OCD research and the three main behavioral models range in their validity. Barbering (rats pulling out the fur of cage-

mates, thought to mimic trichotillomania in human OCD) has strong face validity but lacks predictive and construct validity (Joel, 2006). Marble burying, considered a compulsive behavior and preventable by treatment with SRIs, surprisingly has a low predictive validity because a number of non anti-compulsive drugs also effectively reduce burying. The final behavioral model, the signal attenuation model, is a lever-pressing scheme thought to portray the excessive and inappropriate behaviors comprising the compulsive behavior of OCD. This model has decent validity across the categories mentioned, with selectivity for drugs targeting OCD supporting its predictive validly. Similarities in compulsivity-inducing mechanisms and neural systems support its construct validity, and the behavioral 'compulsive' lever-pressing supports its face validly (Joel, 2006).

**Pharmacological Models and Spontaneous Alternation Behavior**

Pharmacological models are those in which animals exhibit drug-induced OCD-like behaviors. Notable behaviors induced by such drugs include perseveration and indecision, witnessed by changes in the spontaneous alternation behavior of animals injected with an 5-HT1A receptor agonist, as addressed in the present study. Rats naturally show spontaneous alternation behavior (SAB). Divergence from this normal behavior is a model of OCD, thought to mimic the compulsive checking of OCD patients (Joel, 2006). Under normal circumstances, a rat systematically explores its environment fully before returning to any particular location. SAB is disturbed when rats are administered 8-OH-DPAT. Rather than showing SAB, these rats revisit a location repeatedly before continuing on to another area (Yadin, Friedman, & Bridger, 1991).

The reduction of SAB of rats after injection with 8-OH-DPAT is the basis for the current study.

SAB is a widely used method of accessing memory and spatial learning and has been used extensively in OCD research on rats. The impact of injected 8-OH-DPAT within the SAB scheme is a common animal model within the field of OCD research. It is one of the least complicated models to implement in a study as it requires only limited behavioral training in the form of acclimation to the testing devices in addition to the acute drug administration (Joel, 2006). An extensive collection of studies utilizes this model, ranging from the role of ovarian and neurosteroid hormones to lesions and deep brain stimulation (Abdela & Joel, 2012). The present study strives to utilize this model in a new way to investigate the impact of stress on OCD through observations of stress-induced variation in SAB.

The prevention of decreased SAB of rats in the presence of an administered SRI or SSRI treatment leads to a fairly high degree of predictive validity. Predictive validity is increased when the 5-HT1A receptor agonist, 8-OH-DPAT, was found to decrease the alternation of males more than in female rats. Additionally, SRI treatment of 8-OH-DPAT-induced OCD behavior was less successful in juvenile rats than in adult rats. These findings in rats reflect the demographic gender prevalence of OCD in humans (male vulnerability to early-onset OCD) and the weaker response to SRI treatment in children.

Face validity of the SAB model is achieved upon pharmaceutical induction of OCD-like behaviors, since the model focuses on only the behavioral aspects of the disorder. Other forms of validity are less prominent, reflecting the complex brain

circuitry of OCD, which is still not fully understood. Joel (2006) points out that results of the pharmacologically-induced decrease of SAB reflects multiple neurotransmitter systems and psychological processes; since the complex neural systems of this disorder are largely unknown, it can be deduced that more work is needed to establish whether or not other types of validity are supported in the model.

**8-OH-DPAT**

All rats were injected with (+/-)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT) in order to induce compulsive behaviors. 8-OH-DPAT is a selective 5-$HT_{1A}$ receptor agonist, which mimics the effect of serotonin and, like serotonin, is thought to act on both 5-$HT_{1A}$ pre-synaptic and post-synaptic receptors. The mechanism of action of 8-OH-DPAT is complex. At low doses, it preferentially stimulates presynaptic receptors, and because those are autoinhibitory, serotonin cell activity is reduced. However, at higher doses, such as the one used to interfere with spontaneous alternation behavior, 8-OH-DPAT binds to both the inhibitory autoreceptors and the postsynaptic receptors that are distributed throughout the brain, including areas that have previously been shown to relate to OCD-like behavior. Although it is unclear how compulsive behaviors caused by 8-OH-DPAT relate to its effects on 5-$HT_{1A}$ receptors, they are akin to those shown in human OCD patients and can be treated with the same medications used to treat human OCD patients. Thus, this method is an established way of experimentally creating an animal model of OCD.

**The Present Study**

The impact of stress on human OCD is not clearly established. Through using the SAB model, the impacts of stress on OCD-like behavior can be examined. This study sought to link acute stress to increased OCD-like behavior in rats. Additionally, the protective nature of an enriched environment on acute stress was assessed. Subjects that have lived in either a standard or enriched environment were exposed to either predator odor or a control condition, and then examined for OCD-like behavior in the 8-OH-DPAT-induced disruption of SAB model.

## Method

### Subjects

The 32 subjects in this experiment were male, albino, Sprague Dawley rats (from Charles Rivers Laboratories). They were 41 days old upon arrival to the research facility and 62 days old at the beginning of acclimation to experimental conditions. They were housed in pairs and kept in a regular 12-hour light-dark cycle, with experiments conducted during the light cycle. The temperature and humidity of their room was kept constant throughout the experiment. Habituation of the rats to the research facility was allowed to take place for the three days after they arrived. After the habituation period, all rats were handled three times per week and allowed constant unlimited food pellets and water. The Connecticut College Institutional Animal Care and Use Committee, in accordance with the USDA and PHS guidelines described in *The Guide for the Care and Use of Laboratory Animals*, approved all procedures.

### Environmental Conditions

For two weeks before the start of acclimation and testing, the rats lived in either an enriched or standard environment. For the rats living in an enriched environment, the rat cages included a shelter and an exercise wheel. Additionally, these rats had "rat recess," during which twice per week, for 30 minutes at a time, they were allowed to play in groups of four inside a large plastic pool structure filled with wood shavings and various tunnels and toys. The rats in the standard condition were not given "rat recess" and their cages contained only woodchips at the bottom. During the last half of the second week after the arrival of the rats, but prior to the week of acclimation, four fruit loops were placed into each cage so that they could later be used as reinforcement (rather than avoided as a novel food item) during the acclimation and experiment phase.

**Acclimation**

The standard "T" maze was used in the experimental process; a picture of the device can be located in *Appendix A*. The rats had to become acclimated to the structure as well as the action of being placed inside and removed from the device prior to the baseline and testing phase. There were two "T" mazes, and each rat was acclimated to the same maze in which he would later have his baseline and test conducted. Each maze had walls 30 cm high and passageways 13 cm wide, allowing enough room for the rats to fit comfortably and turn around. The stem of the "T" was 26 cm long and the flat top of the "T" measured 90 cm, thus each arm was 45 cm long. One arm of each maze had colorful tape stripes so that the rats could remember each side uniquely. One Fruit Loop in a small bowl was placed at each end of the "T", about 5 cm from the end of the arm. The arms of each "T" maze were equally baited with Fruit Loops and the colors of the

Fruit Loop bowls were kept constant throughout the entire experiment. Clear Plexiglas panels were lowered at some time points to prevent the rat from switching arms after his initial decision had been made. The researchers were careful to align the mazes with the overhead lighting of the laboratory testing room so as not to create shadows within the maze.

The acclimation phase of the experiment began 3 weeks after the rats arrived. During the acclimation phases, as well as during baseline and tests, each arm was equally baited with one Fruit Loop and the researchers conducted their activities at approximately the same time of day. Additionally, the mazes, underlying table, and Plexiglas panels were cleaned with 50% rubbing alcohol between each rat's use in order to minimize pheromone signaling between rat subjects.

The first day of acclimation consisted of the rats, in pairs, being allowed to explore the T-maze for twenty minutes. The second day of acclimation was identical to the first except that each rat was alone for his 20 minutes in the T-maze. The third day of acclimation consisted of each rat spending 5 minutes in each side of the "T," using a clear Plexiglas panel to keep him in each side. The fourth day of acclimation was a repeat of the third day in the hope of making each rat feel comfortable within the arms of the "T." During each of the four days of acclimation, the arms were baited with one Fruit Loop.

On the fifth day of acclimation, a baseline assessment of each rat's spontaneous alternation behavior was recorded. Prior to this day of testing, the rat's food was removed so he would be motivated to seek out the Fruit Loop reward during the assessment. The baseline process consisted of starting the rat within the stem of the "T," preventing movement out of the area by means of the Plexiglas panel. The rat was

positioned with his tail facing the base of the "T" and his face toward the Plexiglas panel. Once the Plexiglas was lifted, a stopwatch recorded the time until the rat made a decision, demarcated by when his hind legs crossed into one side arm of the "T." At this point, the Plexiglas panel was used to prevent the rat from going into any other part of the maze, and he was allowed to eat the Fruit Loop before being lifted out to begin the next trial. The direction (left or right) and latency in decision-making was recorded for a total of 14 trials. If the rat did not leave the starting point in the maze after 90 seconds, it was counted as "undecided," and the rat was picked up, handled/petted for about a minute, and started again on the next trial.

**Testing**

After four days of acclimation and one day of baseline testing, the experimental tests were performed. Each rat was exposed to either the stress odor (TMT) or a control odor (water) for 10 minutes. During this time they were being exposed to the predator odor or control, their behavior was monitored in an activity monitor, as will be later explained. After ten minutes in the observation chamber, each rat was removed and intraperitoneally injected with 2mg/kg of 8-OH-DPAT. After waiting 15 minutes in their home cages to allow the 8-OH-DPAT to take effect, each rat was then tested in the "T" maze, as explained previously for the baseline test.

(For clarification, there was no saline-injected control cohort because the present study sought to examine the effects of stress on rats with OCD, not the effects of stress on healthy rats. The control cohort in the present study was composed of OCD-induced rats

(each injected with 2mg/kg of 8-OH-DPAT) that were not then exposed to the predator odor.)

**Predator Odor Exposure**

Prior to testing their spontaneous alternation behavior in the "T" maze, the rats were exposed to either predator odor (TMT) or control (water) within an activity monitor. The activity monitor consisted of a video camera affixed over a rectangular observation chamber. The observation chamber was a box made of transparent red Plexiglas, thus allowing researchers to see through the walls, yet making them opaque to rats who process the red hue differently from humans. The dimensions of the box were 55 cm long, 29 cm wide, and 30 cm high. The TMT or water was pipetted onto a small piece of filter paper, taped to one of the short sides of the box about 25 cm from the base. A picture of the observation chamber can be viewed in *Appendix B*. The entire activity monitor was set up under a hood to minimize odors or stress pheromones being released beyond the immediate area. The activity monitor divided the observation chamber to demarcate distances from the source of odor. The program, called ANYmaze, allowed researchers to visually partition the area of the chamber and track the specific spatial location of each rat. The program divided the observation chamber into thirds and also created a virtual contact zone surrounding the odor source.

Specific values were recorded for each animal based on his activity and movement within and between these demarcation zones, including the total distance traveled, time in source zone, time in source end, and time in away end. The "total distance" was the measure (in meters) of each rat's horizontal movement throughout the

10 minute exposure to the TMT or control. The "time in source end" was a measure of the amount of time (in minutes) that each rat spent in the third of the observation chamber closest to the odor source, whereas "time in away end" was the time spent in the third farthest from the odor source. The "time in source zone" was a record of the amount of time the rat was very close to, or in contact with, the odor source. Figure 2 in the appendix visually clarifies these virtual demarcations. The amount of freezing by each animal was not recorded because in addition to the activity monitor's inability to differentiate freezing from lack of horizontal distance traveled, reports of freezing observed in rats exposed to TMT greatly vary so this measure seemed less important for our analysis.

In the TMT chamber, 10 µl of undiluted TMT was applied to the filter paper attached to the side of the chamber. In the control chamber, 10 µl of water was placed on the filter paper in the same location in the chamber. Precautions were taken to avoid the TMT smell from being presented to the control rats in this phase by testing the animals on different days and not returning any TMT-exposed animals back to the animal room where untested animals could smell that stressor.

**SAB Scoring**

The scoring of spontaneous alternation behavior (SAB) has been standardized across most if not all of the literature using the SAB model. The way in which the SAB score for each animal is determined is based on a seemingly random number of the first 7 trials during which the rat makes a choice in the T-maze. Then this value of 7 was

divided by the number of times the rat switched direction, thus a perfectly alternating rat would have a SAB score of 1 (7/7).

### Data Analysis

Differences between the data from each rat's baseline and experimental tests were analyzed for differences based on stress condition (TMT vs. water) and living environment (standard vs. enriched). An ANOVA regression and multivariate statistical analyses were then conducted on the data. Animals were omitted from analysis if they had more than one data points above or below two standard deviations from the mean (based on the descriptive statistics of an SPSS linear model).

## Results

### Analysis of Odor Effect

To evaluate the hypothesis that TMT would cause increased OCD-like behavior in the rats, a one-way ANOVA was conducted for a dependent measure of spontaneous alternation behavior (SAB) with type of exposure to odor as the independent variable. As seen in *Figure 1*, there was no significant difference between the Test SAB scores (the number of trials it took subjects to alternate based on the first 7 trials) between the TMT group ($M = 1.44$, $SD = 0.36$) and the no odor group ($M = 1.53$, $SD = 0.31$), $F(1,21) = .46$, $p = .906$.

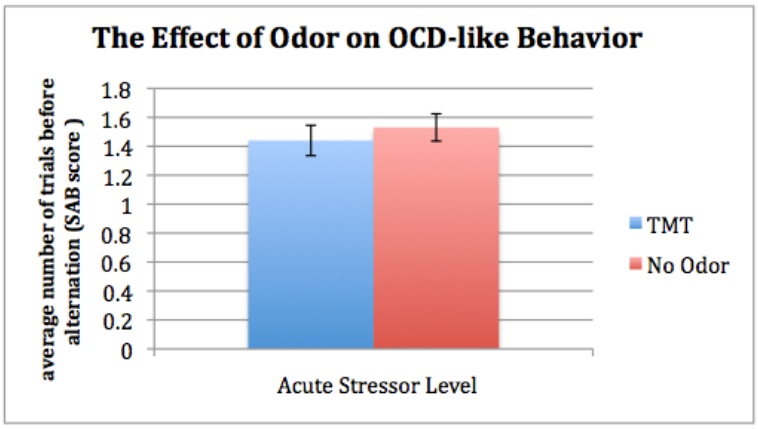


*Figure 1*. Although not significant, rats exposed to no odor demonstrated more OCD-like behavior than did rats exposed to the stressor, TMT.

More importantly, a second ANOVA, using the change in SAB scores as the independent variable with odor condition as the dependent variable, showed no significant difference between the change in SAB scores from baseline to test between either group as seen in *Figure 2*; TMT group ($M = 0.23$, $SD = 0.79$), no odor group ($M = 0.04$, $SD = 0.89$), $F\ (1,26) = .75$, $p = .596$. Thus, any stress induced by the predator odor did not significantly affect the rats' perseverative tendencies.

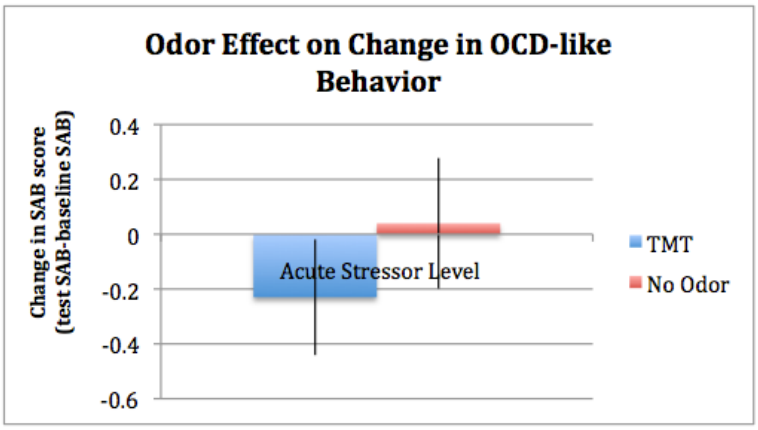


*Figure 2*. The change in SAB score due to presentation of stressor was not significant. In opposition to what was expected, exposure to the stressor resulted in less (yet insignificantly so) OCD-like behavior than in rats exposed to no odor.

Although odor did not significantly affect OCD-like behavior, it was determined from factorial ANOVA analyses that TMT was in fact aversive for the rats. Specifically, three inter-linked dependent variables relating odor condition to the rats' physical location preferences within the observation chamber were all found to be significant when analyzed with odor level as the independent measure. *Figure 3* reflects that the amount of time the rats spent in close proximity to the odor source, a dependent variable termed "source," was significantly less for rats exposed to TMT ($M = 3.76$, $SD = 5.32$) than for rats exposed to a control no odor source ($M = 52.49$, $SD = 36.10$), $F(1,22) = 25.20$, $p < .001$.

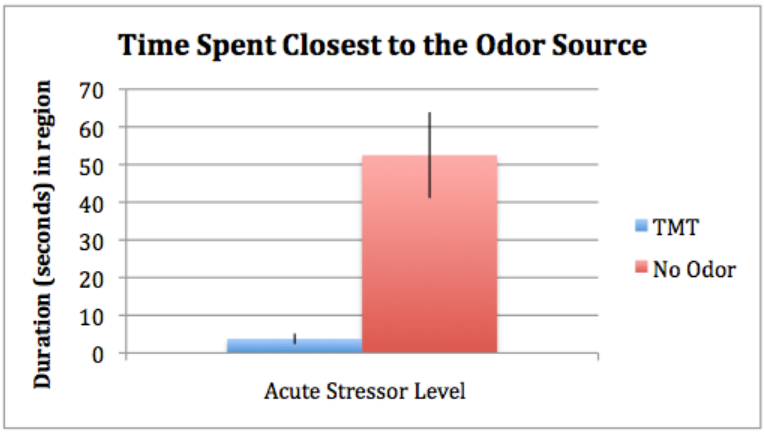


*Figure 3.* Rats exposed to the stressor, TMT, spent significantly less time near the source of the odor when compared to rats exposed to no odor. As expected, the TMT was highly aversive to the rats, but it is hard to know if this is because it was truly a predacious stressor or if the aversion was based on the noxious quality of the synthetic substance.

Likewise, the time rats spent within the third of the observation chamber closest to the source ("close") was significant based on odor with the rats exposed to TMT ($M = 68.07$, $SD = 40.39$), who spent much less time in the "close" area than did rats exposed to no odor ($M = 174.50$, $SD = 88.91$), $F(1,22) = 15.74$, $p = .001$. *Figure 4* reflects this significant finding.

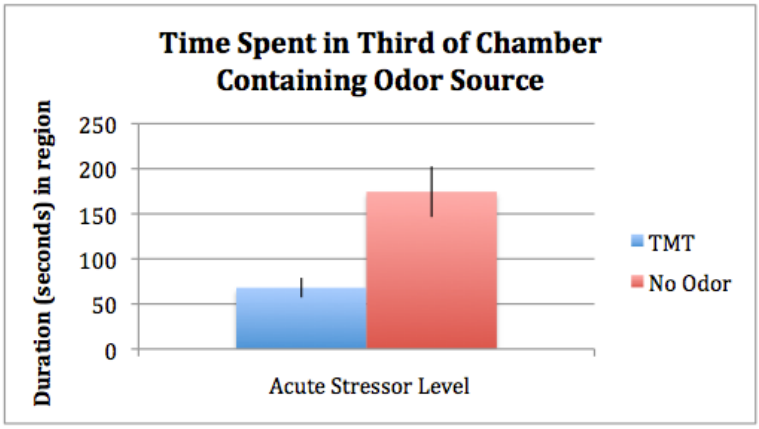


*Figure 4.* As expected and in-line with the previous figure, rats that were exposed to TMT spent significantly less time in close proximity (in the third of the observation chamber closest to the odor source) than did the rats exposed to no odor.

As expected, the rats' time within the third of the observation chamber furthest from the source ("away") reflected their odor condition, with rats exposed to TMT ($M$ = 432.78, $SD$ = 81.19) spending much more time as far way as possible from the odor source than did those in the control group ($M$ = 218.51, $SD$ = 78.47), $F$ (1,22) = 41.754, $p$ < .001. *Figure 5* reflects this significant finding.

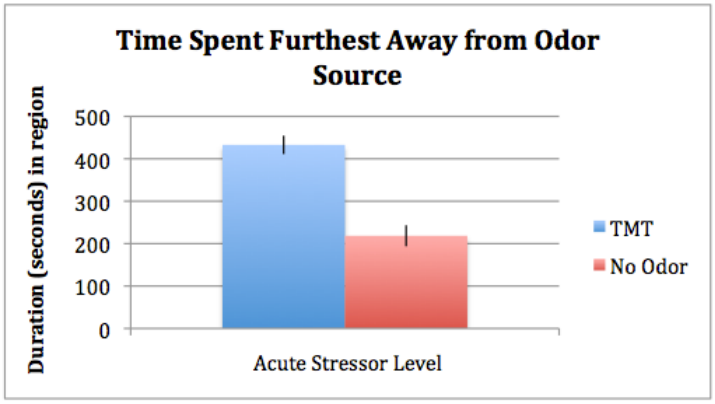


*Figure 5.* As expected, the rats exposed to TMT spent significantly more time in the third of the observation chamber furthest away from the source of the odor, when compared to rats exposed to no odor.

**Analysis of Housing Effect**

To evaluate the hypothesis that housing level would have an effect on OCD-like behavior in that an enriched environment would be protective and thus cause

decreased SAB scores, a one-way ANOVA was conducted for the overall effect of housing level on the change in SAB score from baseline to test. Contrary to the hypothesis, an enriched environment did not prove to be a significantly protective measure in attenuating future stress, although the results were slightly in the expected direction. There were no significant differences between the change in SAB scores from baseline to test between either group; enriched group ($M$ = -0.18, $SD$ = 0.95); standard group ($M$ = -0.09, $SD$ = 0.73), $F$ (1,26) = .289, $p$ = .395. *Figure 6* shows this insignificant finding.

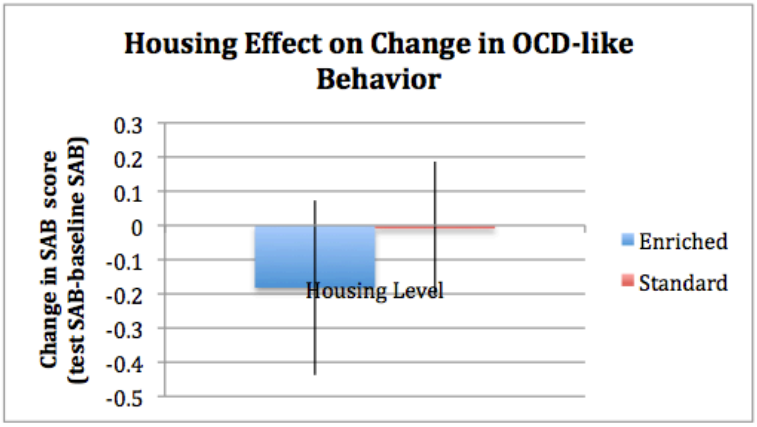


*Figure 6.* Although not significant, rats raised in enriched environments were less susceptible to the effects of future stress, as demonstrated by decreased OCD-like behavior. The larger decrease in SAB score during the test, compared to the baseline, means that, as expected, the enriched environment trended towards being protective towards future stress.

Based on a similar ANOVA, there was also no significant difference between the final Test SAB scores (the number of trials it took subjects to alternate based on the first 7 trials) between the enriched group ($M$ = 1.49, $SD$ = 0.39) and the standard group ($M$ = 1.48, $SD$ = 0.29), $F$ (1,21) = .01, $p$ > .516. *Figure 7* visually reflects this insignificant finding.

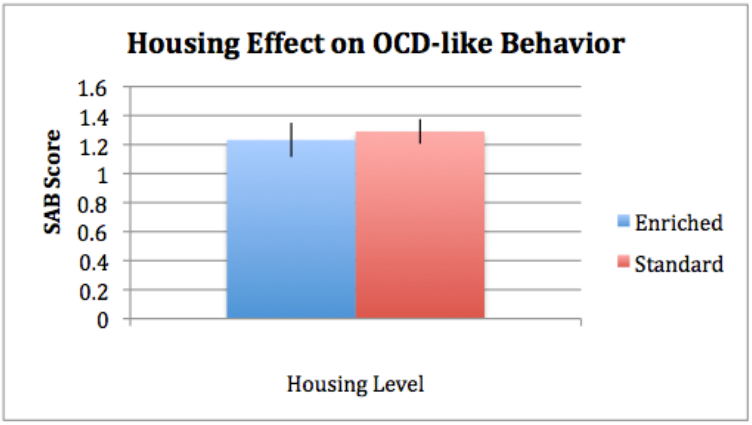


*Figure 7*. Although not significant, the expected trend of the protective nature of an enriched environment was observed. Rats raised in enriched environments had slightly lower test SAB scores than did rats raised in standard conditions.

Based on a various ANOVAs using the independent variable of housing condition and a number of different dependent variables, housing was found to be significant only in the rats' decision time in the T-maze, termed "test latency." *Figure 8* and *Figure 8b* show that rats living in enriched environments ($M = 2.80$, $SD = 1.04$) made quicker choices than did rats housed in standard environments ($M = 5.60$, $SD = 3.95$), $F(1,26) = 6.78$; $p = .015$

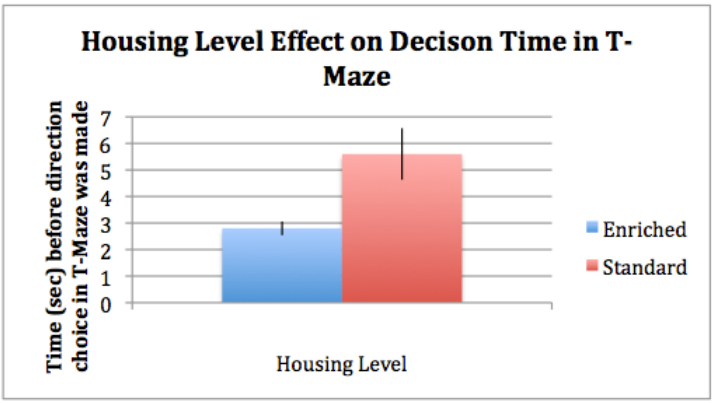


*Figure 8*. The time it took for the rats to make a decision within the T-maze was dependent on the housing level of the rat, with rats housed in enriched environments making faster choices than did those housed in standard conditions.

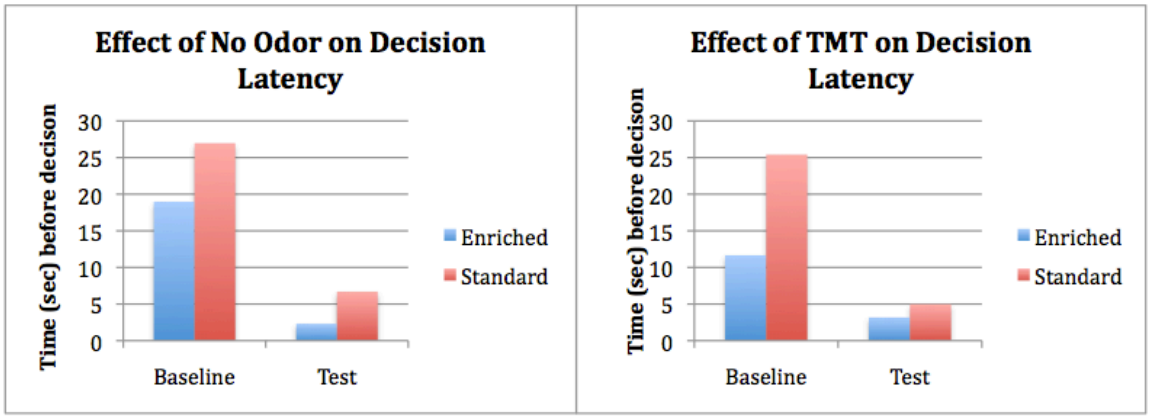


*Figure 8b.* The decision latency within the T-maze was dependent on the housing level of the rat, with rats housed in enriched environments making significantly faster choices than did those housed in standard conditions. The odor level had no significant impact on the decision time of the rats. This finding demonstrates the protective nature of enriched housing.

**Analysis of Multivariate Effects**

An SPSS MANOVA test of between-subjects effects was conducted and three interaction effects were identified. A significant interaction effect was found for the test SAB score, representing the standardized way of measuring spontaneous alternation behavior of the rats within the T-maze. *Figure 9* and *Figure 9b* show that the SAB score was found to vary significantly based on both housing and odor variables, $F$ (1, 22) = 6.33; $p$ = .023. The two highest SAB scores came from the rats housed in enriched environments and exposed to TMT ($M$ = 1.63, $SD$ = 0.48) and those housed in standard environments and exposed to no odor ($M$ = 1.68, $SD$ = 0.27). The lowest SAB scores came from the rats housed in standard environments and exposed to TMT ($M$ = 1.31, $SD$ = 0.19) and those housed in enriched environments and exposed to no odor ($M$ = 1.28, $SD$ = 0.23).

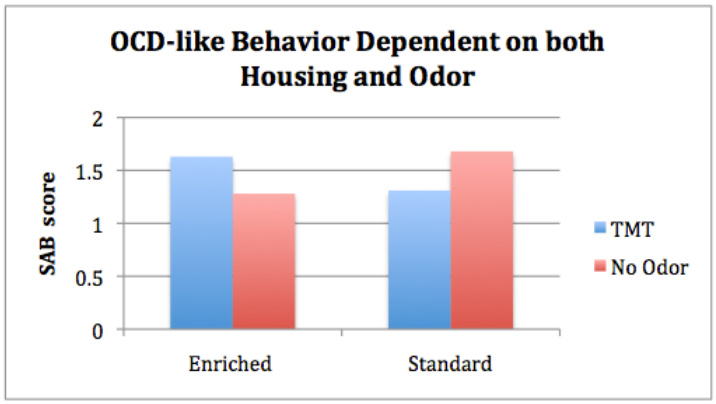


*Figure 9.* An interaction effect was found for the test SAB score based on both the housing and odor variables. The most exacerbated OCD-like behavior was found in rats housed in enriched environments and exposed to TMT and those housed in standard environments and exposed to no odor. The least OCD-like behavior was shown in those rats housed in standard environments and exposed to TMT and those housed in enriched environments and exposed to no odor.

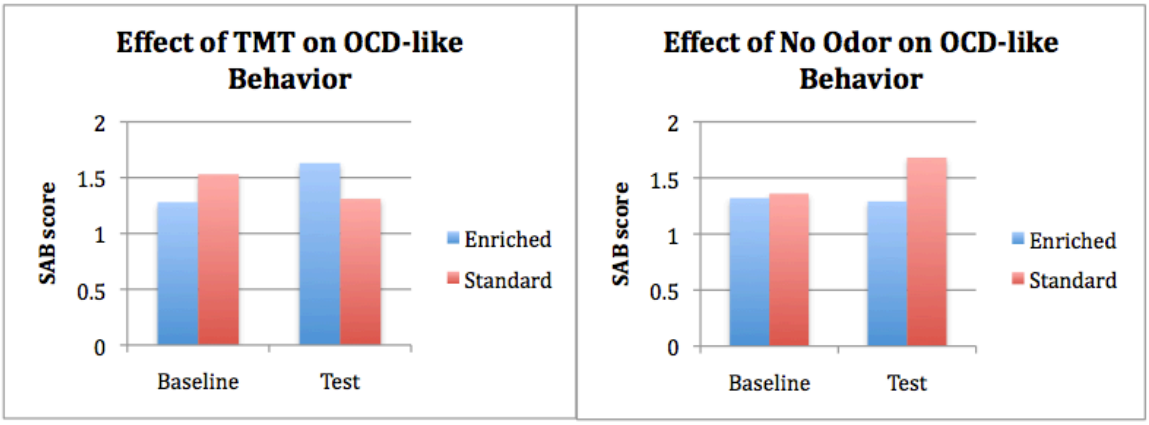


*Figure 9b.* The change in SAB score due to both type of odor exposure as well as housing. Within the rats exposed to TMT, surprisingly those housed in enriched environments exhibited more OCD-like behavior. Within the rats exposed to no odor, those housed in standard environments showed more OCD-like behavior. This finding was a surprise because the enriched housing was expected to protect against increased SAB scores in the rats exposed to TMT and the increased SAB score in the no odor animals was unprecedented because this was meant to be the control measure.

The second significant interaction effect identified was the amount of time spent nearest to the source of the odor while each rat was in the observation chamber. *Figure 10* shows that time spent closest to the odor source was found to vary significantly based on both housing and odor variables, $F$ (1, 20) = 9.71, $p$ = .006. The time spent nearest the source from most to least time nearest the odor source was 1) rats housed in standard environments and exposed to no odor ($M$ = 80.73, $SD$ = 38.08), 2) rats housed in enriched environments and exposed to no odor ($M$ = 24.26, $SD$ = 14.67), 3) rats housed in standard

environments and exposed to TMT ($M$ = 6.04, $SD$ = 6.65), and 4) rats housed in enriched environments and exposed to TMT ($M$ = 2.06, $SD$ = 2.41).

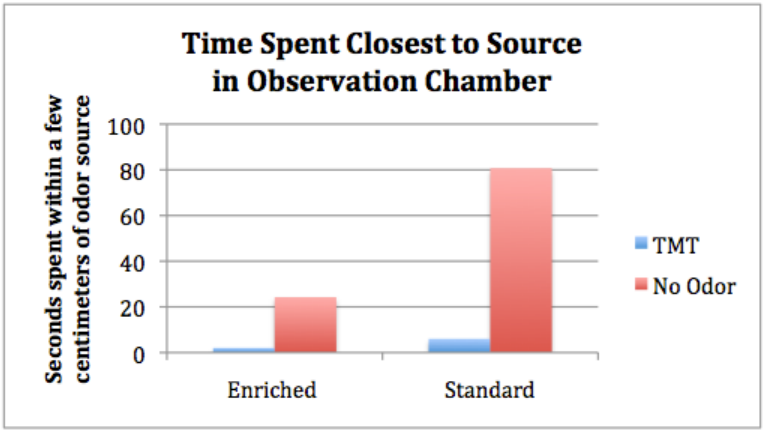


*Figure 10*. There is a significant interaction effect of the time spent closest to the odor source based on both the housing and odor variables.

The ANOVA comparing the location response to TMT as a function of housing condition revealed that standard-housed rats were more responsive to the no odor control compared to those housed in the enriched environment. The researchers thought it would be interesting to determine if there were a direct relationship between time spent in the "source" region of the odor observation box and SAB score in the standard-housed rats. However, a correlation run to assess this relationship was not significant.

*Figure 11* shows another interaction effect; that the total distance traveled by rats within the odor observation chamber was found to vary significantly based on both housing and odor variables, $F$ (1, 20) = 5.74, $p$ = .029. The two groups of rats with the most movement within the chamber were those housed in standard environments and exposed to TMT ($M$ = 22.12. $SD$ = 12.55), and those housed in enriched environments and exposed to no odor ($M$ = 20.53, $SD$ = 8.66). The rats who traveled the least within the chamber were those housed in enriched environments and exposed to TMT ($M$ = 9.85,

*SD* = 6.37) and those housed in standard environments and exposed to no odor (*M* = 12.15, *SD* = 5.10).

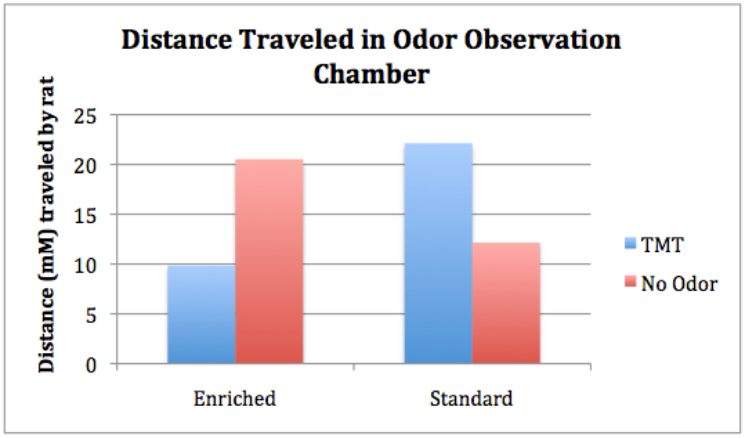


*Figure 11.* There is an interaction effect of the distanced traveled by each rat within the observation chamber based on both the level of housing and the level of odor.

## Discussion

### Effect of TMT

It was expected that a naturalistic stressor would promote OCD-like behavior, and that rats housed in an enriched environment would be protected from the effects of the stressor. The results confirm that the stressor was aversive to the rats: the time the rats spent closest to the odor source was significantly different between groups, categorized by odor. Rats exposed to no odor spent about 13 times longer close to the source than did those exposed to TMT, spending 52.49 seconds within the source box, whereas rats exposed to TMT spent only 3.76 seconds within the source box. Similarly, the time within the third of the box closest to the source was also significantly different between groups with regard to odor as rats exposed to TMT spend 68 seconds close to the source whereas those exposed to no odor spent nearly three times as long, 175 seconds, within the source box. The total time within the odor observation chamber remained constant

for each animal; for that reason it is obvious why the time in the third of the chamber furthest from the source was the highest for the rats exposed to TMT. These findings are consistent with the literature in that rats find TMT either predatory and/or noxious and thus try not to spend excessive amounts of time within the immediate vicinity of the source.

**TMT Not Demonstrated to Significantly Exacerbate OCD**

It was hypothesized that the stress of TMT would increase OCD-like behavior in the rats. Diminished spontaneous alternation behavior is thought to resemble OCD; for that reason increased SAB scores for rats exposed to TMT were expected compared to rats exposed to no odor. Contrary to the hypothesis, acute stress due to TMT exposure was not shown to increase OCD-like behavior in rats. Figure 1 shows that in fact, rats exposed to TMT actually showed marginally less OCD-like behavior than did the rats exposed to no odor. Figure 2 further shows this trend by comparing the test SAB scores to those observed during the baseline test. Exposure to TMT was expected to increase SAB scores, but this hypothesis was not supported by the data. The only justification for this finding is if the rats exposed to TMT were somehow more vigilant and thus resilient to OCD-like tendencies than were those not exposed to TMT. However, this is an unsupported theory that does not follow findings in the literature nor make sense when thinking back to the human condition of OCD. Results of the present study likely were unsupportive of the hypothesis for a number of possible reasons. First of all, 8-OH-DPAT did not seem to induce OCD-like behavior to the extent that is observed in published studies. Additionally, other possibilities include i) TMT did not induce stress

or ii) stress does not exacerbate OCD. No previous studies have been conducted linking TMT-induced stress to the SAB model so it is difficult to compare direct findings of the present study to findings in the literature.

**i) Issues with using TMT as a stressor**

As discussed in the introduction, there is much controversy over the reliability of TMT as a naturalistic stressor versus as a highly noxious stimulus. Statistically significant results of the present study verified that the rats did avoid the TMT, as shown in Figures 3, 4, and 5. Rats exposed to TMT spent significantly less time near the source of the odor and more time away from it, when compared to rats exposed to no odor. Although the rats' avoidance of TMT was clear, it is impossible to know if their avoidance of it in the observation chamber was due to stress or simply its noxious quality. Given this uncertainty, it is impossible to conclude that predator stress has no effect on SAB because perhaps TMT alone is an ineffective predator stressor. Although there are valid reasons against its use, as described in the introduction, cat odor may have been a better option to use because it is unquestionably a predator odor.

**ii) The chance that stress does not increase OCD-like behavior**

OCD, both in its behavioral manifestations and physiological basis, is highly impacted by stress so it is unlikely that this explains why TMT did not increase OCD-like behavior. Lin et al. (2007) demonstrated that stress exacerbates OCD behaviors, and Lord et al. (2011) demonstrated that increased cortisol levels, caused by stress, increase the severity of OCD symptoms. Additionally, structural and activation changes within

the OCD brain highlight areas that are also associated with stress. Giedd et al. (2000) describe how brain areas such as the basal ganglia are shown to be larger in OCD than in non OCD brains and when additional stress is added, maladaptive behaviors result from already overwhelmed brain areas (such as the PFC) that struggle to continue normal neural signaling. Neurotransmitters including serotonin and dopamine are also linked to the stress response and OCD-like behavior. Brain area-specific serotonin release and resulting behaviors in rats under stress have not been found consistently in research, yet the role of decreased serotonin in OCD across many brain areas is a well-established finding (Goddard et al., 2008; Lucky, 1998). Goddard et al. (2008) relate the behavioral decline of OCD patients to changes in the OCD-loop brain areas dependent on serotonin. Dopamine is largely associated with habit-forming behaviors due to its reward and pleasure pathways. Dopaminergic neurons increase their rate of firing if an animal is under stress, leading to increased compulsion and perseveration, both maladaptive behaviors defining OCD.

There is extensive evidence supporting the link between increased stress and increased OCD-like behavior; for that reason it is unlikely that the present study accurately managed to model the effects of stress on OCD-like behavior, resulting in no significant effect. There likely is an effect of stress on the OCD-like behavior of rats, but the SAB model failed to capture such an effect for a number of possible reasons. As previously explained, TMT may not have been a stress-inducing stimulus to the point where it would have affected SAB behavior or, as explained next, perhaps the whole SAB model is not the correct one to use when assessing the effects of stress on OCD.

**Failure to Induce Statistically Significant OCD**

Another problem encountered in the present study was the failure to induce significant OCD-like behavior in the rats. Figure 2 shows the change in SAB score between the baseline and test dates; it is of concern that the induction of OCD on the test date in fact *decreased* the SAB score instead of elevating it. A lower SAB score is interpreted as less OCD-like behavior, which makes no sense as the serotonin agonist is expected to increase the OCD-like behavior of rats, which is shown by increased perseveration represented by an increased SAB score.

Prior to this study, the researchers conducted a pilot study where the dose of 8-OH-DPAT (2mg/kg) was confirmed as the best dose; it was the lowest effective dose, causing significant OCD-like behavior yet not being overpowering to the point where disadvantageous physiological side effects impacted the rats' movement within the maze. Additionally, the dose used in the present study is consistent with what is found across the literature. However, for some unknown reason, in the present study, this well-established dose did not seem to induce OCD as significantly as was expected, as shown in the overall lack of significantly increased SAB score. Alternatively, perhaps the manipulations of this study were responsible for the diminished observed OCD-like behavior. It was hypothesized that SAB scores would increase with TMT exposure as increased SAB scores reflect increased OCD-like behavior; the lack of such an increase is intriguing, yet unlikely to be a reliable trend. For the present study, perhaps the rats should have had OCD induced twice, once before the experimental manipulations of odor and housing were applied, and once after. This way, the effectiveness of the drug could be verified, and then further differences between the second baseline (with 8-OH-DPAT)

and the test (also with 8-OH-DPAT) would have shown differences in SAB based only on the dependent measures being tested (odor and housing effects) and not also based on the induction of OCD-like behaviors.

**No Significance of Housing Level on OCD-like Behavior of SAB**

In terms of the housing manipulation, the hypothesis of an enriched environment being protective toward future stress was not statistically supported, but did seem to trend in the hypothesized direction. Figure 7 demonstrates that rats housed in enriched environments seemed to be marginally resistant to future stress based on slightly (not significantly) lower SAB scores. This was the expected trend as there is a large, solid body of literature supporting the protective effects of enriched environments towards stress (Nazar & Martin, 2011; Takuma et al., 2011). Our findings trended in the anticipated direction yet were not significant. One concern that may be responsible for this outcome was the small sample size used. In fact, when each of the 8 rats' scores were counted twice (thus hypothetically doubling the sample size of each condition to be 16 animals) the findings became significant with the *p*-value falling below the .05 significance level. Although not a significant finding in the present study, repeating the study with more animals likely would show an enriched environment to be protective against future stress. Eight animals per experimental group seemed a bit low considering the observed variation within group, but this sample size was found to be typical of many studies utilizing the SAB model.

**Effect of Housing on OCD-related Decision Making Latency**

The only significant finding within the housing variable was that of the latency of the rats' decisions in picking an arm of the T-maze, with rats housed in enriched environments making quicker decisions. Rats housed in standard conditions took over twice as long to make their decision (2.49 seconds for enriched rats vs. 5.11 seconds for standard rats) as did rats in enriched environments. This finding, shown in Figure 8 and 8a, was not anticipated but makes sense based on the understanding of an enriched environment. As explained in a study by Sztainberg, Kuperman, Tsoory, Lebow, and Chen (2010), decreased anxiety is a well-established result of an enriched environment, noted in rats by their reduced levels of emotionally-related measures including freezing. Although the amount of freezing by each rat was not documented within the T-maze, perhaps the standard rats had increased latencies because they were freezing more than were the rats raised in enriched environments. In general, because rats living in enriched environments are more active and more highly stimulated on a daily basis than are rats living in standard conditions, it is justifiable why they would perhaps self-soothe if under stress and be more decisive within a maze. The decision-making time of rats between housing conditions was significant and reflected success in capturing behavioral changes based on housing condition, with the decreased anxiety level of the enriched environment rats likely responsible for the decreasing response time, compared to the rats housed in standard conditions.

The decreased latency in decision making of rats housed in enriched environments is possibly one way of demonstrating the protective effects of an enriched environment on OCD-like behavior. Although the actual decisions the rats made were not indicative of them having OCD, the extended time they took to make the decisions

could lend itself to demonstrating that OCD was actually induced. OCD is often referred to as a decision-making disorder whereby people with OCD often struggle to make decisions to the point where making such choices impacts normal life. A study by Foa et al. (2003) determined that even concerning low-risk decisions, people with OCD spent significantly more time deliberating their choice than did people without OCD. For this reason, increased latency could be a manifestation of OCD-like behavior in the rats in the T-maze. The rats housed in enriched environments made faster decisions when exposed to the TMT stressor, thus showing less OCD-like behavior than did rats housed in standard environments. The baseline latencies were not found to significantly differ based on housing, which supports the idea of its protective ability toward OCD, rather than just decreasing decision time for all rats regardless of OCD-induction.

This finding makes sense when the OCD associated neurobiological abnormalities are investigated. A study by Cavedini, Gorini, and Bellodi (2006) highlighted that many of the brain areas that play a role in OCD, such as the orbitofrontal cortex, basal ganglia, and thalamus, also are important to executive functioning and cognitive-behavioral flexibility, which markedly impact decision making ability. More specifically, a later article by many of the same authors (Cavedini et al., 2001) pinpointed the ventromedial prefrontal cortex deficiencies akin to those in people with brain damage to this area, and linked this region to the decision-making deficit associated with OCD. Furthermore, Sachdev and Malhi (2005) link decision-making difficulties in OCD with the amygdala and limbic system, as well as other areas of the "OCD-loop," explaining that abnormalities in relation to the history of reward likely impact decision-making ability in people with OCD. Although the test latency and not the baseline latency was found to

significantly differ based on housing, no significance was found based on the difference in latency times between the test and the baseline, which perhaps again sheds light on some internal problems with the SAB model.

**Interaction Effects Showing Protective Nature of Enriched Environments**

Neither housing nor odor alone had individual effects on the SAB scores of the rats, but a significant interaction effect between the two was found. Figure 9 shows the interaction effect of both housing and odor on OCD-like behavior, as recorded in each animal's SAB score. The most exacerbated OCD-like behavior was found in the rats housed in enriched environments and exposed to TMT and in those housed in standard environments and exposed to no odor. The least OCD-like behavior was shown in those rats housed in standard environments and exposed to TMT and in those housed in enriched environments and exposed to no odor. Within the enriched animals, the observed trend makes sense because TMT exposure was expected to exacerbate OCD-like behaviors. However the reverse trend within the standard animals, of the TMT exposure decreasing OCD-like behavior, was a surprise finding. Although not supported in the literature, perhaps the stress induced by the TMT caused the rats to be hyper-vigilant within the T-maze, thus showing less OCD-like behavior than did their no-odor counterparts, as reflected in their decreased SAB scores.

The second significant interaction effect was the amount of time spent nearest to the source of the odor during the time each rat was in the observation chamber. Figure 10 shows that time spent closest to the odor source was found to vary significantly based on a combined effect of the housing and odor variables. As previously found, rats exposed

to no odor spent the most time closest to the source, but those housed in standard conditions spent significantly more time close to the source compared with rats housed in enriched environments. This finding likely just shows that the rats housed in standard conditions were more interested in the novelty of the parchment paper (source of TMT odor or control) than were the rats housed in enriched environments, who were more accustomed to novel objects. This finding is not especially pertinent to the goals of the current study, but did indicate that the housing conditions had a behavioral effect on the rats,

Another unexpected finding was that the total distance traveled by rats within the odor observation chamber was found to produce an interaction effect, varying significantly based on the combined effect of housing and odor. As shown in Figure 11, the two groups of rats with the most movement within the chamber were those housed in standard environments and exposed to TMT and those housed in enriched environments and exposed to no odor. The rats who traveled the least within the chamber were those housed in enriched environments and exposed to TMT and those housed in standard environments and exposed to no odor. Brillaud, Morillion, and de Seze (2005) found that exploratory behavior of enriched animals increased compared to those housed in standard conditions, so this finding was a bit surprising. Perhaps these results show that the TMT exposure excited the rats raised in standard environments, thus making them more active and likely more anxious than was true of the rats raised in enriched environments.

This finding would be supported in the literature as a component of the protective nature of enriched environments. There is a large body of evidence demonstrating decreased stress responses in rats housed in enriched versus impoverished environments,

in particular the physiological indicators of decreased corticosterone and adrenaline responses to the induced stress (Benaroya-Milshtein, et al., 2004; Moncek, Duncko, Johansson, & Jezova, 2004). Additionally, a study by Segovia, Arco, Blas, Garrido, and Mora (2007) found that levels of dopamine produced were significantly lower in rats raised in enriched environments compared to those raised in standard conditions. Dopamine is a sympathetic nervous system activating neurotransmitter, thus influential in the flight or fight response of an animal, so decreased amounts of dopamine under stressful conditions can show the protective nature of enriched housing. The findings for the no-odor rats in this interaction effect were less relevant to our study but further support the successful implementation of enriched vs. standardized housing. Out of the no-odor rats, those housed in enriched environments were more accustomed to exploring whereas rats housed in standard environments were accustomed to more sedentary lifestyles.

When results of the distance interaction effect are viewed alongside the previously explained interaction effect of time spent closest to the odor source (Figure 10), perhaps the rats housed in standard conditions and exposed to TMT were exhibiting the largest stress response possible: attempted escape. Of the rats exposed to TMT, that all spent the most time away from the odor source, those housed in enriched environments were perhaps able to self-soothe (and travel significantly less), whereas the rats housed in standard conditions were not able to self-soothe or find alternative coping skills and thus were frantically running all over the side of the observation chamber furthest from the TMT. These findings are consistent with a large body of literature supporting the protective effects of enriched environments toward future stress. Larsson,

Winblad, and Mohammed (2002) found distinct stress response effects based on the housing variable of enrichment. This study found that the protective effects of enriched housing toward future stress as well as the long-lasting nature of the enrichment effects.

The study by Larsson et al. (2002) also found that pre-exposure to stress has a decreased negative effect on enriched animals compared with standard animals. Although not directly related to this experiment as the odor exposure was the only acute stressor, the testing procedure in the T-maze could be stressful, especially when under the influence of an anxiety-inducing drug. This procedure could explain why the enriched housed animals were able to make significantly faster decisions (Figure 8) and directional choices that trended toward significance (Figures 6 and 7) than were the animals housed in standard conditions. Based on the behavioral effects of housing and odor from this interaction, the protective nature of an enriched environment, toward the predator odor stressor as well as the stress associated with the induction of OCD-like behavior, is likely supported.

## Possible Directions for Future Experimentation

### Concerns with Sample Size and Analysis Criteria for Present Study

Results from the present experiment were not as straightforward or clear-cut as expected. One explanation for this outcome was the small sample size of each experimental group ($n$=8) as well as issues involved with collecting data. The AnyMaze software used to record the rats' behavior while in the observation chamber was not working correctly for the first 4 rats tested, so the observational analysis for these rats had

to be discarded. Additionally, animals were omitted from analysis if they had two data points above or below two standard deviations from the mean (based on the descriptive statistics of an SPSS linear model). Through this process, four rats were omitted from the statistical analysis, but it is hard to know if an increased sample size would change the number of omitted animals. Similar studies in the literature report groups ranging from about 6 to 12 animals (with some studies reporting as few as 4 or as many as over 20 animals); after any rat was dropped a given group in the present study, the number of animals fell toward the lower range of the typical sample size.

**Equivalent Rat Stress to Human Stress**

In relation to exacerbated OCD in humans, the stressor used in the current study was not a fair match to the types of stress humans normally feel. Predator odor stress for a rat signifies a life-threat whereas human stressors are almost always non-life threatening and social in nature. Even repeated exposure to TMT may not have created a similar stress situation to that experienced by humans, although repeated exposure may have been better than a single exposure. Social defeat stress, considered most similar to the types of social stress humans experience, is a type of stressor that is possible to implement in rat experiments. However, social defeat stress entails a complicated experimental process including training to create complex social dynamics between the rats and is most often used to mimic bullying in humans (Vidal, Buwalda, & Koolhaas, 2011). Although social defeat stress likely would have been the best type of stress to use in matching the type of rat stress to human stress, it was not feasible for this experiment.

**Problems with the SAB Model**

**SAB Scoring**

When recording and analyzing the data for the present study, three different techniques of SAB scoring were undertaken. The first way was the standardized approach described in the methods section. The second way of SAB scoring was based on the maximum number of trials a rat could successfully complete (make a decision for) out of 14 possible trials. The SAB score was then determined by the total number of trials completed by the number of alternations throughout the whole test. This approach was thought to result in a more accurate SAB score, as it was the average number of trials taken to alternate as in the standardized SAB scoring system, yet usually included the average of more than just 7 trials. However, in some instances, the rat did not successfully complete 7 trials when given 14 opportunities; then this animal was still counted in the SAB analysis, whereas with the standardized SAB scoring the rat would be disqualified and not used in further analysis.

The third way SAB scores were calculated in the present study was including the "undecided" trials in the final count. Thus 14 trials was always the numerator, and this total divided by the number of alternations was the calculation used for the third method of SAB scoring. The problem with this method was that it, perhaps unfairly, took into account the number of undecided trials the same way that repeated lefts or rights were recorded. In many instances, the rat seemed to fatigue over the course of the test so that by the end, he did not make a decision for multiple trials and instead spent all 90 seconds in the stem of the "T."

Having conducted the SPSS analysis for all three SAB scores for each animal, the only significant findings regarding SAB were found using the standardized method of SAB scoring. Although selecting the standardized method may seem like an arbitrary way of accounting for spontaneous alternation behavior, it is likely the best way of recording decision-based OCD-like behavior in rats.

## Other Possible Issues/Errors

### Variation Within Group

There was a great deal of variation across the individual rats' data points within each larger trend. Although this is the experimental nature of studies, it was a concern that for such a small sample size, this variation could have impacted the analyses and thus the results of this study. Additionally, in one instance, data within the same experimental measures differed depending on which day they were tested. No cause could be identified for these differences. Each cohort was staggered by one week in terms of when they were tested, so there could be environmental factors that unknowingly impacted the results. For example, perhaps the way in which the different cohorts were raised and transported by Charles River Laboratories varied. Other possible sources of difference were the differences in personnel who handled them each week as well as possible unperceived environmental differences over time in the rooms in which the rats were housed and tested.

### Animal Models of OCD

**Unknown Role of Serotonin in OCD**

One of the most obvious concerns with studying OCD, especially when trying to justify the use of one model over another, is the still widely unknown role of serotonin. OCD is thought to be primarily a serotonergic disorder, but there still is little concrete evidence to prove what actual role this neurotransmitter has in the pathogenesis of OCD. Additionally, although the overall clinical utility of SRIs and SSRIs are understood, their exact neural sites and mechanisms of therapeutic action are still widely unknown. It is important to note the fact that nearly half of human OCD patients are not successfully treated by SRIs or SSRIs, further complicating the already seemingly mysterious nature of serotonin on the OCD brain. The lack of effect of high frequency stimulation on rats within the SAB model, as later explained, has brought the concern of the unknown serotonergic role to light in terms of this animal model. The usefulness of the SAB model previously hinged on the assumed similar serotonergic manipulation between the animal model and actual human condition. However, because neither the animal nor human serotonergic system is fully understood in terms of what precise dysfunction(s) lead to OCD, creating well-established and reliable animal models will remain a challenging task.

**Validity and Applicable Use of the SAB Model**

Although every animal model of OCD has unique strengths, none to date is able to satisfy all three types of validity; face validity, construct validity, and predictive validity. The SAB model, using 8-OH-DPAT to induce OCD-like behavior, is one of the

most frequently used animal models of OCD, but some current literature is beginning to make researchers think it may not be as good as a model as first thought. Albelda and Joel (2011), who have analyzed the breadth of studies using all sorts of animal models of OCD, point out that recent findings further question the validity of the SAB model. Specifically, high frequency stimulation (HFS) was shown not to be therapeutic in rats administered 8-OH-DPAT, whereas this is a very effective therapeutic approach in human OCD, thus detracting from the SAB model's predictive validity. Additionally, lesions to the orbitofrontal cortex of rats administered 8-OH-DPAT have been found not to impact the OCD-like behavior as measured by the SAB model. This brain area is thought to be a key component of OCD; for that reason the finding that its functioning has no impact on the SAB model, greatly detracts from the SAB model's construct validity. That being said, SRIs and SSRIs have been demonstrated to be extremely effective in treating the induced OCD-like behavior brought on by the SAB model. Additionally, Umathe, Vaghasiya, Jain, and Dixit (2009) highlight that multiple drugs proven ineffective in treating human OCD (like imipramine, venlafaxin, moclobemide, diazepam and buspirone) were likewise ineffective in the SAB model, so the strong drug-related predictive validly of the model must not be overlooked. Despite its weaknesses, the 8-OH-DPAT based SAB model is still considered one of the best, especially in terms of its strong predictive validity with regard to most drugs used to treat OCD in humans.

Although the 8-OH-DPAT based SAB model has fairly strong pharmacological strengths, perhaps it was not the appropriate model to use for the behavioral approach of the present study. Albelda and Joel (2011) as well as Umathe et al. (2009) further point out that not only is decreased alternation common in many neurological and psychiatric

conditions, but such decreases in SAB can be the behavioral results of multiple interwoven neurotransmitter systems and/or psychological processes. For this reason, it is suggested that the use of the SAB model is more suited for testing the anti-compulsivity nature of drugs rather than as a means of studying the neural mechanism of OCD.

**Future Studies**

If this experiment were to be repeated, a larger number of animals would be needed to determine the significance of the odor and housing effects on SAB individually as well as their combined effect. Additionally, the pros and cons of using TMT over cat odor would have to be further assessed. It also might be beneficial to test similar hypothesizes using a different animal model of OCD. Albelda and Joel (2011, 2012) explain that a whole host of genetic models of OCD are being developed, with increasingly more validity than previous genetic models. However, in addition to increased cost and level of experimentation, there are still many unknowns concerning these strains of genetically modified mice so exploring the effects of stress on such animals is completely uncharted territory and perhaps is best suited for more standardized animal models. Alongside the 8-OH-DPAT induction of SAB, the other most popularly used animal model of OCD is the marble-burying test. Marble burying (both in duration of burying as well as total number of marbles buried) has been shown to vary proportionately to the amount of anti-OCD drug administered, thus marble burying has been identified as an anxious, obsessive-compulsive behavior in rodents. In addition to comparative ease and cost-effectiveness to run, the marble-burying test is well established as a means of testing a

measure of OCD unrelated to spontaneous alternation. The SAB model may have failed to capture some OCD-like behavior that was not related directly to alternation patterns; for that reason perhaps running the marble-burying test alongside a re-run of the current SAB study with more subjects would be the next steps in determining the effects of stress on OCD.

## Conclusion

Although the results of the present study were largely inconclusive in terms of the SAB model, there is much room for future exploration both within the SAB model and other models to explore the role of stress in OCD. Despite no concrete findings supporting the effect of stress of OCD-like behavior as measured by SAB, there were significant interaction effects of both odor and housing. These findings did support the hypothesis of protective effects of enriched environments toward later stress, including both the rats' behavior within the observation chamber as well as time latency within the T-maze. Future studies could be conducted to insure the stressor effect of TMT as well as the successful induction of OCD, as would likely be observable in statistically significant differences between the two levels of each variable. To advance the field, perhaps combining the SAB model with another more behavioral-based model, such as the marble-burying test, might be a better, more well-rounded approach to examine the effect of stress on OCD.

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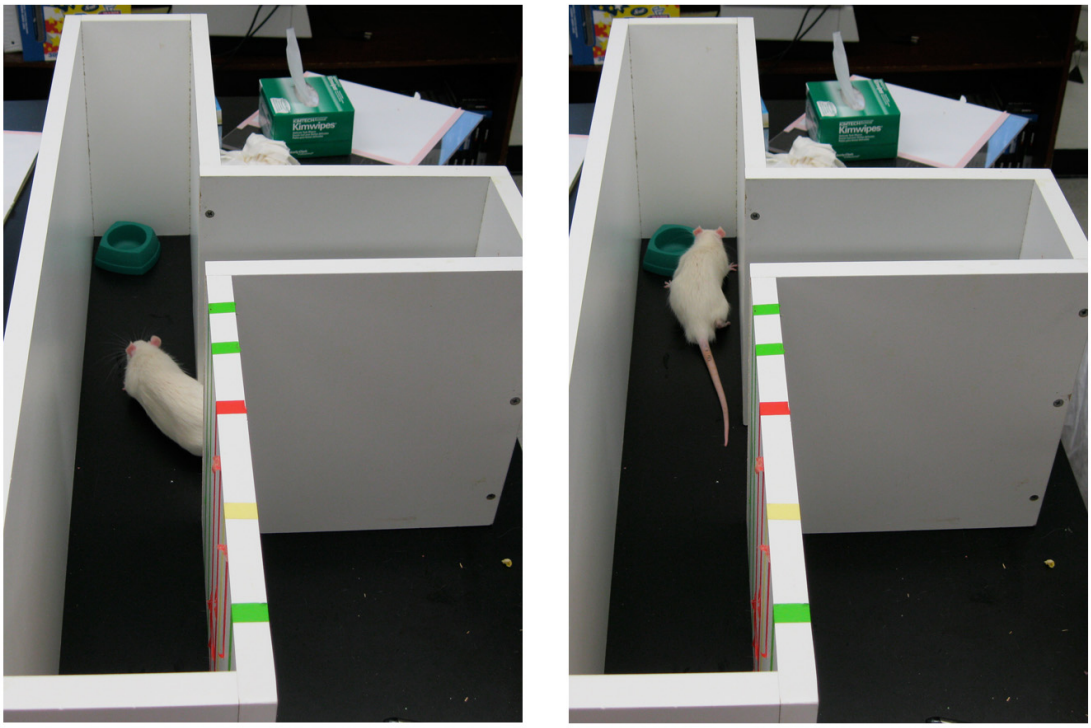
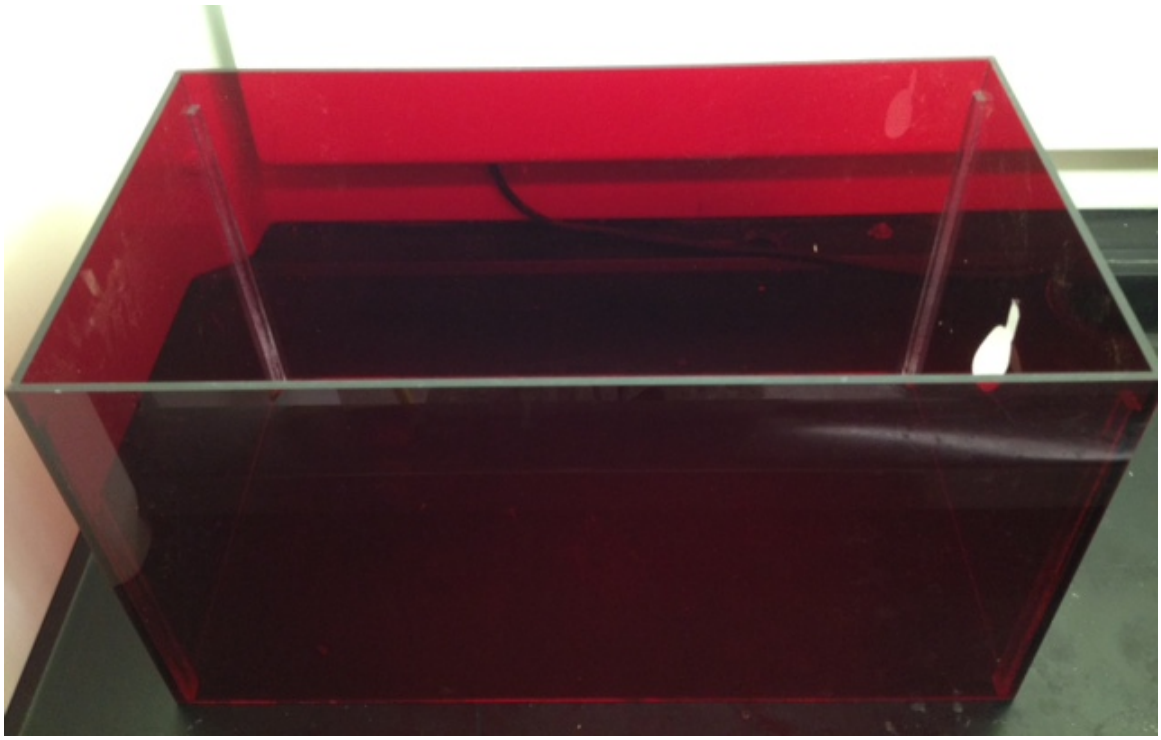
Appendix A




"T" Maze used for SAB testing

Appendix B



Observation chamber used for odor exposure