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Effects of Early Life Stress on Anhedonia and Striatal Dopamine Concentration Depends on Variation in catechol-O-methyltransferase (COMT) Genotype

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Preface

My work in the Applied Research Externship Program in Clinical Outcomes at Astor Services for Children and Families in Rhinebeck, NY, combined with my volunteering experience with the children in Astor's residential program, has had a profound and gratifying effect on me as well as a strong influence on my current research interest. Astor Services for Children and Families provides services and treatment for children whose parents are having trouble caring for them for various reasons. Many of the children enrolled in Astor's services have experienced various forms of abuse or have witnessed abuse towards their parents or violence in their communities. Last spring at Astor, I contributed to a project on clinical outcomes of children and families enrolled in Astor's satellite outpatient programs in Bronx, NY. I was initially shocked at the sheer number of children enrolled in Astor's programs, as the current number of case files exceeded ten thousand.

In my anecdotal observations, there seemed to be a high occurrence of depression, anxiety, self-harm, and suicide attempts for children who had experienced childhood abuse or trauma. This sobering observation compelled me to further investigate potential effects of childhood trauma on behavioral and psychological outcomes. My reading of the literature leading up to this project has revealed some potential mechanisms that may be at play leading to adverse outcomes, and specifically, led me to investigate gene x environment interaction factors in early life trauma that may modulate vulnerability to depression.

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Abstract

Depression is a serious, costly, and heterogeneous disorder for which no one genetic determinant has been identified. Research has shown that stress, and subsequent hypothalamic-pituitary-adrenal (HPA) axis dysregulation, is a significant predictor of depression, and one particular stressor that has been linked to vulnerability to depression and HPA axis dysregulation is early life trauma. Due to the heterogeneity and complexity of depression, it is likely that specific gene-environment interactions play a role in the development of depression. Interaction between catechol-O-methyltransferase (*COMT)* Val^{158}Met variants with specific environmental factors can potentially increase vulnerability to depression. The present proposed experiment examines the interaction between *COMT* genotype and the effects of early life stress in a maternal separation paradigm on behavioral and neurological factors in a rodent model of depression. Outcome measures include anhedonia-like behavior, as indexed by performance on a sucrose consumption test, and striatal dopamine concentration, a neurological measure of depression. I hypothesize that across genotype, stress leads to decreased sucrose consumption and decreased striatal dopamine concentrations. However, the functional *COMT* Val^{158}Met polymorphism will interact with stress such that these results are most pronounced among stressed Met/Met rats, as compared to Val/Met rats and Val/Val rats. These results may have implications for treatment of depression in *COMT* polymorphic individuals, who appear to be more sensitive to stress and more vulnerable to developing depression.

Chapter 1: Literature Review

According to the World Health Organization (WHO), depression is currently the leading cause of disability worldwide as measured by years lived with disabilities (YLDs), affecting 350 million people of all ages (WHO, 2015). Depression is characterized by serious impairments in physical and emotional domains (Rost, 2009) such as depressed mood, feelings of worthlessness, anhedonia, and changes in appetite and sleep (APA, 2013). At any given time point, about 6% of the US population meet the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria for depression (Penninx et al., 2013). Depression is costly, accounting for 4.3% of the global burden of disease (WHO, 2013). It is expected that by 2020, depression will be the second leading cause of disease globally, as measured by disability adjusted life years (Chapman and Perry, 2008). Furthermore, a recent meta-analysis has demonstrated that depression increases the risk of overall mortality and is associated with increased medical morbidity (Penninx et al., 2013).

Symptom presentation in depression is relatively heterogeneous, due to the categorical nature of DSM diagnosis. For example, some diagnostic features of depression, which were previously referred to as subtypes in the DSM-IV (APA, 2000), include depression with melancholic features, depression with catatonic features, and depression with atypical features (APA, 2013). Depression with melancholic features is marked by anhedonia and loss of pleasure, as well as despair, weight loss, excessive guilt, psychomotor agitation or retardation, early-morning awakening, and/or depression that is worse in the morning (APA, 2013). Depression with catatonic features is marked by catatonic behavior, such as stupor, catalepsy, waxy flexibility, mutism, negativism, posturing, odd mannerisms, stereotypy, agitation, grimacing, echolalia, and/or echopraxia (APA, 2013). In contrast, depression with atypical features is

marked by mood reactivity and weight gain, hypersomnia, leaden feelings of the arms and legs, and/or interpersonal rejection sensitivity (APA, 2013). Because cases of depression vary in terms of symptomatology, it may be beneficial to approach investigation into the mechanisms and etiology of depression by examining specific aspects of depression in a dimensional, rather than a categorical approach to the disorder.

Anhedonia and Depression

As mentioned above, anhedonia is a core symptom of depression, associated with decreased subjective experience of pleasure and reduced interest in previously enjoyed activities (APA, 2013; Naranjo et al., 2001). A recent review suggests that anhedonia is an aspect of depression distinct from mood disturbance, and that is comprised of motivational (such as diminished drive to achieve a goal) and consummatory components, such as diminished participation in activities that were once considered pleasurable, or decreased consumption of palatable substances in rodent models of depression (Argyropoulos and Nutt, 2013). These two components of anhedonia map onto Berridge and Robinson's (1998) incentive salience hypothesis, in which reward is comprised of two components: "wanting," of a stimulus and "liking" or the hedonic value associated with a stimulus.

The presence of anhedonia, or the decreased experience of pleasure and decreased motivation to partake in pleasurable activites, in depression may predict worse outcomes with treatment. One study evaluating 811 adults with moderate to severe depression demonstrated that the presence of anhedonia symptoms (such as loss of interest, diminished activity, lack of enjoyment) predicts poorer outcomes in response to selective serotonin reuptake inhibitor (SSRI) or noradrenergic antidepressant treatment (Uher et al., 2012). Similarly, anhedonia has been shown to predict poorer recovery among adolescents with SSRI treatment-resistant depression

(McMakin et al., 2012). These findings denote the difficulties in treating depression, as clinical symptoms manifest differently across individuals and signaling via various neurotransmitters with varying neural pathways may subserve different aspects of the disorder. Furthermore, these findings indicate that further research on the mechanisms of anhedonia and outcomes associated with it, as well as on markers of vulnerability to depression with anhedonia, are necessary in order to inform treatment approaches and improve depression treatment outcomes.

Etiology of depression

As mentioned previously, depression has proven to be a complex and heterogeneous disorder, such that it may result from a variety of causes (Duman, 2014; Malki, 2014) and comprises "multiple disorders with overlapping symptoms and diverse etiologies" (Hamon, 2013). According to a meta-analysis on the epidemiology of major depression, heritability of the disorder ranges between 31% and 42% (Sullivan et al., 2000). These heritability estimates allow for a large environmental influence in the etiology of depression. Twin studies reviewed in the above article demonstrate the complexity of the etiology of depression, as both environmental and genetic influences together were required for the disorder to occur (Sullivan et al., 2000). It is likely that specific gene-environment interactions play a role in the development of depression, and dysregulation in multiple neurological pathways lead to depression, contributing to the considerable heterogeneity of the disorder (Sullivan et al., 2000).

Importantly, in a recent meta-analysis of genome-wide association studies (GWAS) for major depressive disorder, including 18,759 subjects, no locus reached genome-wide significance (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013). In other words, within this study with a very large sample size, no one genetic determinant could be identified for major depressive disorder. These results (Major Depressive

Disorder Working Group of the Psychiatric GWAS Consortium, 2013) support the hypothesis that depression is comprised of multiple disorders and is extremely heterogeneous in its etiology and neural pathways (Hamon, 2013; Penninx et al., 2013). Therefore it is important to study depression in terms of gene-environment interactions in order to parse the heterogeneous origins of the disorder and identify the mechanisms of the disorder.

One way to investigate gene-environment interactions in the etiology of depression is to look at anhedonia as an endophenotype of depression, as anhedonia is a central aspect of depression and, "vulnerability markers that are inherent to a psychiatric disorder have been proposed as 'intermediate' phenotypes of 'endophenotpes'," (Antypa et al., 2013). Furthermore, in terms of gene-environment interactions, the effect of the gene on the endophenotype might be more readily and robustly demonstrated than the effects of the gene on a full, complex phenotype (Antypa et al., 2013). In other words, gene-environment interactions may therefore be best demonstrated on an endophenotype rather than the complex set of phenotypes that make up clinical depression.

Chronic stress and depression

One major theory of the etiology of depression suggests that chronic stress and subsequent hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis is causally involved in the development and maintenance of depression (McEwen, 2002; Roy & Campbell, 2013; Guerry and Hastings, 2011; Tarullo and Gunnar, 2006). The HPA axis (see Figure 1) is a physiological system that includes the hypothalamus, pituitary gland, and adrenal glands, and comprises a major component of the body's stress response, culminating in the release of mineralocorticoids such as aldosterone, and glucocorticoids, such as cortisol (McEwen, 2002). The HPA axis functions to help the body react to a stressor, such that it may be able to fight or

flee, as well as to signal that the stressor is gone and thus the stress response should be ended (McEwen, 2002). The hypothalamus, pituitary gland, and adrenal glands form a circuit that ultimately promotes the release of corticosteroids (mineralocoids and glucocorticoids). This system normally comes into play upon noticing the presence of a stressor and is initiated by the release of epinephrine and norepinephrine. This prompts the release of corticotropin-releasing factor (CRF) from the hypothalamus to signal the pituitary gland to release adrenocorticotropic hormone (ACTH) into the bloodstream (McEwen, 2002; Seaward, 2013). When the ACTH reaches receptors in the adrenal glands, this prompts the adrenals to release corticosteroids, such as cortisol (McEwen, 2002; Seaward, 2013).

The major function of the corticosteroids released in the stress response is to prepare the body for a fight or flight reaction in response to a serious stressor, promoting survival in a threatening situation. Mineralocoids function to modulate fluid retention and regulate sodium levels, and glucocorticoids such as cortisol function to increase blood pressure and heart rate, convert glycogen into glucose in preparation for energy expenditure, and mobilize stored fats (Seaward, 2013). Once the situation is no longer threatening and the stressor has gone, acetylcholine is released in order to "turn off" the arousal effects of the HPA stress response and conserve energy (Seaward, 2013). Within the HPA axis, cortisol and epinephrine have self-regulatory pathways (McEwen, 2002), which activate to shut off the stress response once the stressful event is over. One way in which the HPA system can become dysregulated is if these self-regulatory pathways fail, and the stress response remains on, or in a hyper-responsive state (McEwen, 2002).

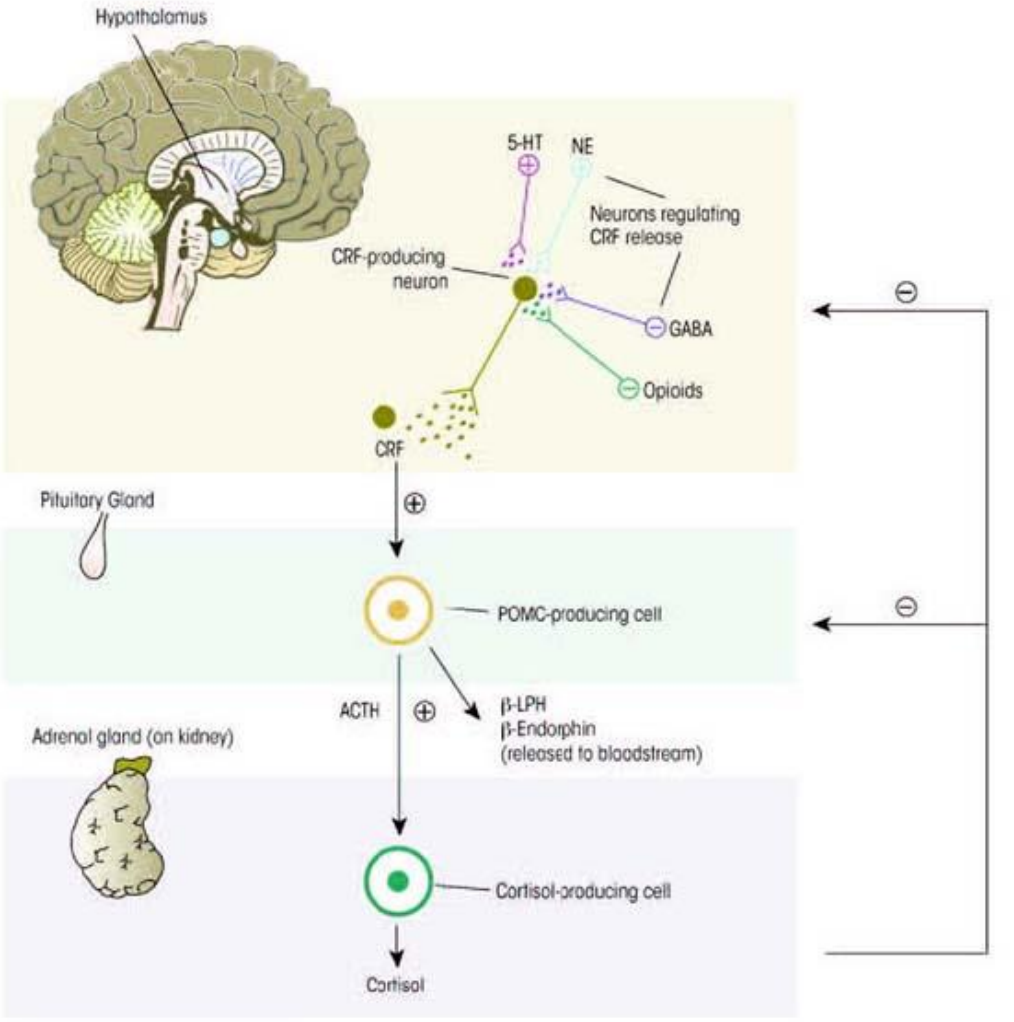

Figure 1. Schematic of the hypothalamic-pituitary-adrenal (HPA) axis stress response. The hypothalamus releases CRF, which prompts the pituitary gland to release ACTH into the circulation. ACTH prompts the adrenal glands to release glucocorticoids, including cortisol. Adapted from Stephens and Wand, 2012.

Chronic stress can lead to dysregulation of the HPA axis, and this can lead to detrimental effects to both physical and mental health (McEwen, 2002). This has implications for depression, as it has been proposed that dysregulation of the HPA axis, particularly in the form of hyper-

reactivity of the system, increases vulnerability to depression (McEwen, 2002) by making certain people "more susceptible to the depressogenic effects of stress" (Guerry and Hastings, 2011). This view implicates the HPA axis as having a potential mechanistic role in depression, which may be influenced by both environmental and genetic factors. Indeed, longitudinal research reviewed in a recent article shows support for the hypothesis that overall HPA dysregulation precedes and predicts development of depression later in life (Guerry and Hastings, 2011). These results are indicative of HPA axis hyperactivity, and stress exposure across the lifespan has been shown to cumulatively increase risk for depression (Vinkers et al., 2014). Thus dysregulation of the HPA axis and subsequent hypercortisolism is causally associated with increased vulnerability to depression.

Early Life Trauma and Depression

One significant stressor that has been identified in the scientific literature as a risk factor for depression is childhood trauma (Neigh et al., 2009; Briere and Jordan, 2009; Heim et al., 2008). Recent review articles have demonstrated the correlation between early life trauma and increased risk of depression as well as the connection between early life trauma and HPA axis dysregulation (Neigh et al., 2009; Briere and Jordan, 2009; Heim et al., 2008). Childhood physical and sexual abuse, and depression have been linked to over-production of cortisol and a hyper-responsive HPA axis (McEwen, 2002; Read et al., 2005). Furthermore, in cases of trauma or chronic stress, the negative feedback loops in the HPA axis become dysregulated, and thus the stress response, and the production of cortisol is not "turned off" in the absence of a stressor (Lee and Sawa, 2014).

The association between early life trauma and increased risk of depression appears to be specific to childhood trauma, such as physical, sexual, or emotional abuse, and is not

demonstrated between other adverse childhood events (such as parental divorce) and depression (Hovens, et al., 2010). Patients with depression have been shown to have experienced significantly more severe traumatic events than control participants (Bandelow et al., 2013) and individuals with a greater number of previous major depressive episodes have experienced greater childhood trauma exposure than control participants (Morris et al., 2014). In addition, early life trauma has been connected to increased emotional reactivity in response to stressors later in life (Shapero et al., 2014).

Animal models support a connection between emotional reactivity and early trauma. For example, in a recent rodent experiment modeling maternal neglect, a form of childhood trauma, it was revealed that rat pups exposed to low levels of maternal care as opposed to high levels of maternal care were more susceptible to stress and anhedonia-like behaviors (Henningsen et al., 2012). Rats exposed to low maternal care also exhibited higher levels of corticosterone following exposure to chronic mild stress (Henningsen et al., 2012). These findings (Henningsen et al., 2012) suggest that early life stress leads to HPA axis dysregulation and increased susceptibility to anhedonia-like behavior.

Interestingly, early life trauma seems to have pronounced effects on outcomes relating to psychopathology, as compared to trauma in adulthood. This seems to be because the brain is in a critical stage of plasticity and development at this time period, and the specific effects of stress on the brain and behavior "depend on the timing and duration of exposure," (Lupien et al., 2009). Furthermore, the brain is particularly sensitive to the effects of stress during the early life period (Lupien et al., 2009). For example, one study of healthy humans has shown that early life stress causes alterations in regions of the brain associated with emotion, as evidenced by decreased amygdala volume and cortical thickness in the rostral anterior cingulate cortex (Korgaonkar et

al., 2013). In addition, studies involving rodent models of early life stress demonstrate similar effects. Early life stress has been demonstrated to interfere with brain development, affecting the structural and functional plasticity of the medial prefrontal cortex, contributing to anxiety-related behaviors in adolescent rats (Chocyk et al., 2013). The effects of early life stress have also been shown to impair coping abilities when faced with stressful challenges in adult rats (Horovitz et al., 2012).

As early life trauma has been shown to lead to negative outcomes relating to psychopathology in humans and in rodent models (Lupien et al., 2009; Korgaonkar et al., 2013; Chocyk et al., 2013; Horovitz et al., 2012), recent research has examined variations in maternal care in rodent models of early life trauma (Carlyle et al., 2012; George et al., 2010). George et al., (2010) designed a model of early life neglect in which mice pups were exposed to maternal separation with early weaning (MSEW). They found that one strain of mice exposed to MSEW exhibited increased anxiety, hyperactivity, and behavioral despair, as seen in the open field test, elevated plus maze, and forced swim test (George et al., 2010). Another rodent model of early life trauma is periodic postnatal infant-mother separation, in which mouse pups are separated from their mothers for 3 hours per day during the first 10 days of life (Murgatroyd and Spengler, 2011). When implemented with mice, this paradigm induced elevated corticosterone levels, increased endocrine responsiveness to stress, altered negative feedback of the HPA axis, and reduced coping abilities in response to stress (Murgatroyd and Spengler, 2011). In addition, another study examining the effects of stressors in juvenility in rats found similar behavioral results, and also that stress during this period of the lifespan alters the maturation of the limbic system (Tsoory et al., 2008). This finding is significant because the limbic system is integral to emotion processing, and thus dysregulation of this system could underlie a predisposition to

stress-related behaviors and psychopathology. Taken together, these studies suggest that early life trauma is particularly harmful to the developing brain, conferring vulnerability to various psychopathologies.

Dopamine Dysregulation and Depression

Early pharmaceutical treatments for depression, beginning in the 1950s, reflected monoamine theories of depression (Mulinari, 2012). These theories generally postulate that depression occurs as a result of insufficient levels of monoamines, such as norepinephrine or serotonin. Two common classes of compounds used to treat depression and increase levels of catecholamines in the brain at this time were monoamine oxidase inhibitors (MAOIs), such as Iproniazid, and tricyclic antidepressants (TCAs), such as Imipramine (Mulinari, 2012; Shorter, 2009). Later on, the serotonin hypothesis of depression came to prominence, postulating that depression occurred as a result of too little serotonin in the brain. In the late 1980s and 1990s, selective serotonin reuptake inhibitors, including Prozac, were marketed, with seemingly great therapeutic success (Mulinari, 2012; Shorter, 2009).

Current theories of depression tend to focus on serotonin and serotonin pathways, but because depression is so heterogeneous a disorder, multiple pathways must be implicated in order to explain the varied causes and presentations of the disorder. Recent research has shown that current treatments for depression with SSRIs are not sufficiently effective, as determined by remission rates (Sinyor et al., 2010), suggesting that investigation of other mechanisms of depression is needed. Dopamine may be a good candidate for alternate mechanistic hypotheses of depression, as it mediates movement, affect, emotion, cognition, and reward (Haenisch and Bonisch, 2011). Dopamine has been extensively researched for its potential role in anhedonia (associated with the inability to experience pleasure) and depression (Argyropoulos and Nutt,

2013).

As mentioned above, dopamine has been shown to play a critical role in motivation and reward seeking behavior (Haenisch and Bonisch, 2011; Salamone et al., 2007) and it has been shown to play a role in the expectation of pleasure (Sharot et al., 2009). It has been postulated that the neural pathways subserving reward processes include the mesolimbic, nigrostriatal, and mesocortical dopamine reward pathways (Argyropoulos and Nutt, 2013). Specifically, it has been suggested that the striatum (part of the nucleus accumbens in the basal ganglia), which receives dopaminergic input from the ventral tegmental area, is critical to reward processing (Nestler and Carlezon, 2006). Berridge and Robinson (1998) put forth the incentive salience hypothesis, which posits that reward can be separated into components of "wanting" and "liking," and these processes are mediated by different neural systems. This hypothesis suggests that dopamine mediates the "wanting" but not the "liking" (the hedonic value of a stimulus) component of reward (Berridge and Robinson, 1998; Berridge, 2007). Furthermore, the "dopamine-related neural systems that mediate 'wanting' interact with hedonic and associative learning components…to produce the larger composite process of reward" (Berridge and Robinson, 1998). Thus dopamine is an important factor in the neural processing of reward, but it is likely not responsible for the entire reward response.

Recent evidence has shown that dopamine dysregulation is associated with anhedonia (Argyropoulos and Nutt, 2013). For example, Sarchiapone et al. (2006) examined dopamine transporter (DAT) binding in a sample of depressed patients with anhedonia as compared to healthy controls, and found that depressed patients showed lower DAT binding in the striatum. Furthermore, dopamine reuptake transporter knockout (DATKO) mice show decreased anhedonia, or an antidepressant-like behavior (Haenisch and Bonisch, 2011), implicating

dopamine dysregulation in depression. Dysregulation of dopamine, and thus of reward-relevant functions mediated by dopamine, can be a component of depression in some cases, and in fact, some current antidepressant drugs such as buproprion, target dopamine transporters in order to increase synaptic dopamine (Porcelli et al., 2011).

Evidence for a Gene-Environment Interaction in the Etiology of Depression

Research has shown that depression develops as a result of the interaction between adverse environmental factors (stress) and genetic predisposition to vulnerability to stress (McGowan and Kato, 2008). Furthermore, gene-environment interactions seem to be an important factor in the adverse outcomes of humans who experienced various forms of early life trauma (Briere et al., 2009). Research has provided support for gene-environment interactions in depression in association with multiple different genes, indicating again the heterogeneity of the disorder. Genes that have been identified in gene-environment interactions in depression include variants of 5HTTLPR, which codes for a promoter region of the serotonin transporter in humans (Colman and Ataullahjan, 2010; Banducci et al., 2014), MAOA, or monoamine oxidase A (Jabbi et al., 2007), FKBP5, or FK506 binding protein (Holz et al., 2015), and COMT, or catechol-O-methyltransferase (Norrholm et al., 2013; Antypa et al., 2013), among others. These genes code for various enzymes or proteins that have implications in depression and specifically in neural pathways associated with depression.

Catechol-O-methyltransferase, or COMT, is critical for the catabolism of dopamine (Norrholm et al., 2013). The *COMT* gene is located on chromosome 22q11.2 (Antypa et al., 2013). A *COMT* polymorphism that has been studied extensively is the $Val^{158}Met$ polymorphism, which has been linked to mental illness (Norrholm et al., 2013). The $Val^{158}Met$ polymorphism is a single nucleotide polymorphism that codes for a substitution of valine for

methionine at codon 158 (Lachman et al., 1996; Antypa et al., 2013). Wild type (WT) *COMT* genotype codes for valine (Val), and the variant codes for methionine (Met). Thus individuals homozygous for the wild type allele have the Val/Val genotype. Heterozygotes are Val/Met, and those homozygous for the variant allele are Met/Met. Having one or more copies of the Met allele is associated with decreased COMT enzyme activity (Lachman et al., 1996), and thus increased dopamine levels in the brain. In contrast, the wild type Val/Val genotype is associated with increased COMT activity (Lachman et al., 1996) and thus decreased dopamine levels in the brain. These findings suggest that the Met/Met genotype might be associated with disorders that are potentially connected with too much dopamine/ dopamine activity (such as schizophrenia), while the wild type Val/Val genotype might be associated with disorders potentially connected with too little dopamine activity (such as depression). However, results have shown conflicting evidence for an association between frequency of one allele or genotype and depression, suggesting that there is no direct association between *COMT* genotype and depression (Antypa et al., 2013).

While there have been mixed results on which variant confers vulnerability to depression, most studies investigating *COMT* do not account for gene-environment interactions (Antypa et al., 2013). Recent human association studies have provided evidence that having the Met/Met genotype in combination with having experienced a significant life stressor is associated with depression (Mandelli et al., 2006; Opmeer et al., 2010; Antypa et al., 2013). This gene-environment interaction suggests that the Val158Met polymorphism is implicated in increased sensitivity to stress, through modulation of the HPA axis, as the Met/Met genotype is associated with greater HPA reactivity (as indicated in salivary cortisol levels of individuals with the Met/Met genotype as compared to those with the Val/Met or Val/Val genotypes) and stress

sensitivity (Walder et al., 2010). This association of Met/Met genotype (in combination with stress) and increased vulnerability to depression is contrary to earlier findings which suggest that having the Met allele leads to decreased COMT activity, increased dopamine activity, and susceptibility to disorders such as schizophrenia (Lachman et al., 1996). Taken together, evidence thus far suggests that the Met allele might be a predictive factor for depression in combination with the presence of stressors (Antypa et al., 1996), but not as an independent predictor of depression.

Animal Models of Depression

In order to test for the effects of stress on COMT activity and vulnerability to depression across *COMT* genotype, the proposed study will utilize an animal model of depression. One reason for investigating this putative gene-environment interaction in animals arises from ethical and practical considerations, as genes cannot be manipulated in humans, nor can humans be randomly assigned to significant early life trauma. Animal models are a useful method for implementing genetic manipulation studies and for investigating gene-environment interactions. Studies involving genetic manipulation as well as environmental manipulation allow for greater investigation as to the neurological mechanisms of the disorder. In addition, being able to actively manipulate genotype in a rodent model of depression allows the investigator to control for potential confounding variables that would be present in a human association study sample, such as socioeconomic status, age, education level, health history, level of social support, and type and number of stressful events experienced. Finally, using genetically manipulated rodents will ensure that all of the experimental subjects are genetically identical except for the target gene. Thus differences across genotype can be attributed to the polymorphic variant in this type of rodent study, whereas if human subjects were involved, they would differ on a wide variety of

genes and thus it would be hard to attribute outcome differences to the target genetic polymorphic variant.

As mentioned previously, in humans, symptoms of depression include depressed mood, anhedonia, changes in weight, sleeping patterns, and psychomotor function, loss of energy, feelings of worthlessness, impaired concentration, and recurrent thoughts of death (APA, 2013). Some of these symptoms can be studied in animals in order to investigate behavioral and neurophysiological changes and mechanisms associated with depression. Rodent models are a useful avenue for studying the neurobiological mechanisms of the disorder, however many of the above symptoms, particularly those relating to feelings of worthlessness, mood, and thoughts of death, are impossible to model on laboratory animals (Yan, et al., 2010). For this reason, specific behaviors associated with depression and behavioral symptoms of depression are modeled in animals.

While depressed mood cannot be modeled in animals, anhedonia, or lack of interest or pleasure in all or most activities (APA, 2013), can be targeted. Anhedonia in animals is demonstrated by decreased preference for sucrose or saccharin (Yan, et al., 2010). Anhedonia-like behavior in animals might be considered an endophenotype of depression, effectively acting as an intermediary phenotype or characteristic of the disorder (Antypa et al., 2013). To test anhedonia, animals are exposed to acute or chronic mild stress and their consumption of palatable sugar solutions are measured (Preti, 2011). Decreased consumption of the substance has been shown to result from chronic mild stress (Grippo, et al., 2003) and chronic social isolation (Grippo et al., 2007), and is understood to be representative of anhedonia, and thus depression.

Taken together, it is useful to study neurological mechanisms and behavioral aspects of depression in relation to the *COMT* Val^{158}Met polymorphism in rodents. Firstly, due to ethical concerns, genes cannot be manipulated in humans and it may not be ethical to implement a chronic stress paradigm in humans as well. It certainly would not be ethical enroll human children in order to study the putative interaction between genetics and the effects of early life stress. Second, in order to control for a multitude of potential confounds and ensure that results occur as a result of the experimental manipulation alone, a rodent model is a more suitable method. Examining the effects of genotype and early life stress on behavioral and neurological aspects of depression can thus be done best by utilizing rodent samples.

Summary of Rationale and Hypotheses to be Tested

Depression is a serious, costly (WHO, 2015), and heterogeneous disorder (Duman, 2014; Malki, 2014) for which no one genetic determinant has been identified (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, 2013). Research has shown that stress (and subsequent HPA axis dysregulation) is a significant predicting factor of depression, (Colman and Ataullahjan, 2010; McEwen, 2002; Roy & Campbell, 2013) and one particular stressor that has been linked to vulnerability to depression and HPA axis dysregulation is early life trauma (Neigh et al., 2009; Briere and Jordan, 2009; Heim et al., 2008). Animal models are a useful way to look at models of depression and trauma and to begin to investigate gene-environment interactions in neural mechanisms of depression.

Evidence suggests that depression develops as a result of the interaction between adverse environmental factors (stress) and genetic predisposition to vulnerability to stress (McGowan and Kato, 2008). Due to the heterogeneity and complexity of the disorder, it is likely that specific gene-environment interactions play a role in the development of depression, and dysregulation in

multiple neurological pathways lead to depression (Sullivan et al., 2000). Dopamine may be a good candidate for alternate mechanistic hypotheses of depression, as it mediates movement, affect, emotion, cognition, and reward (Haenisch and Bonisch, 2011), and these processes are impaired or altered in depression. Further investigation of *COMT*, a gene responsible for catabolism of dopamine, may provide information on one specific mechanism in the etiology of depression. Interaction between *COMT* Val^{158}Met variants with specific environmental factors can potentially increase vulnerability to depression. More research in this vein is necessary because previous studies on COMT and depression have not been conclusive, nor do they account for gene-environment interactions (Antypa et al., 2013).

The present study aims to investigate the mechanisms of this relationship (COMT Val^{158}Met polymorphism X early life stress) in order to further understand this one particular putative route to depression. Specifically, the proposed experiment aims to investigate the effects of stress on COMT and dopamine function across different *COMT* genotypes in a rodent model. In the present study, I will examine the effects of stress on COMT and dopamine function, and a behavioral measure of depression (anhedonia). Based off of the findings from human association studies (Mandelli et al., 2006; Opmeer et al., 2010; Antypa et al., 2013), I hypothesize that stressed Met/Met *COMT* genotype rats will show greatest vulnerability to depression across all measures, and that nonstressed Met/Met *COMT* genotype rats will show the least vulnerability to depression across all measures. I predict that Val/Met COMT genotype rats will show an intermediary effect of the met allele X environment interaction.

Chapter 2: Method

Subjects

COMT variant breeder Wistar rats will be purchased from Charles River Genetically Engineered Model Services, Charles River, Kingston, NY. Subjects will be Wistar rats, because this strain has been used previously in research examining *COMT* expression and dopamine concentration (Schendzielorz et al., 2013). Rats pregnant with Val/Val pups (*n*=4), Val/Met pups (*n*=4), and Met/Met pups (*n* =4) will be housed singly in opaque polycarbonate birth cages (48 cm X 38 cm X 20 cm) lined with 4 cm of paper bedding in a temperature controlled room (22°C) with a twelve hour light/dark cycle (lights will be turned on at 8:00 AM), following the methods of Hancock and Grant, 2009. Food and water will be available to the pregnant rats as desired. Pregnant rats will be checked twice daily for parturition (at 9:00 AM and 5:00 PM). Day of birth will be considered postnatal day (PND) 0. *COMT* genotype will be confirmed in two pups from each litter, by isolating genomic DNA from a tail snip sample (<5mm) collected on PND1, followed by PCR-based allelic discrimination (TaqMan genotyping assay, Applied Biosystems, Irvine, CA).

Postnatal Treatment

Following the methods of Hancock and Grant (2009), on PND 1, each breeder rat will be removed from the birth cage and placed individually in a clear polycarbonate holding cage (45 cm X 24 cm X 20 cm) lined with 2 cm of paper bedding and given open access to food and water. Pups will be removed from the birth cage by a gloved experimenter and placed by litter in individual plastic, paper towel lined containers (26.5 cm X 18.5 cm X 11 cm). These containers will be placed in an incubator (in a room separate from that where the mother rats are housed) at 55%–58% humidity and nest-like temperatures of 33–34°C on PND 1 to 6 and 31–32°C on PND

7 to 14 (Hancock and Grant, 2009). On PND 1, each of the litters will be culled to 10 pups (5 males and 5 females). Half of total Val/Val, Val/Met, and Met/Met litters will be randomly assigned to the stress condition (*n*=60), and the other half of the litters will be assigned to the no stress (control) condition (*n*=60) (see Figure 3).

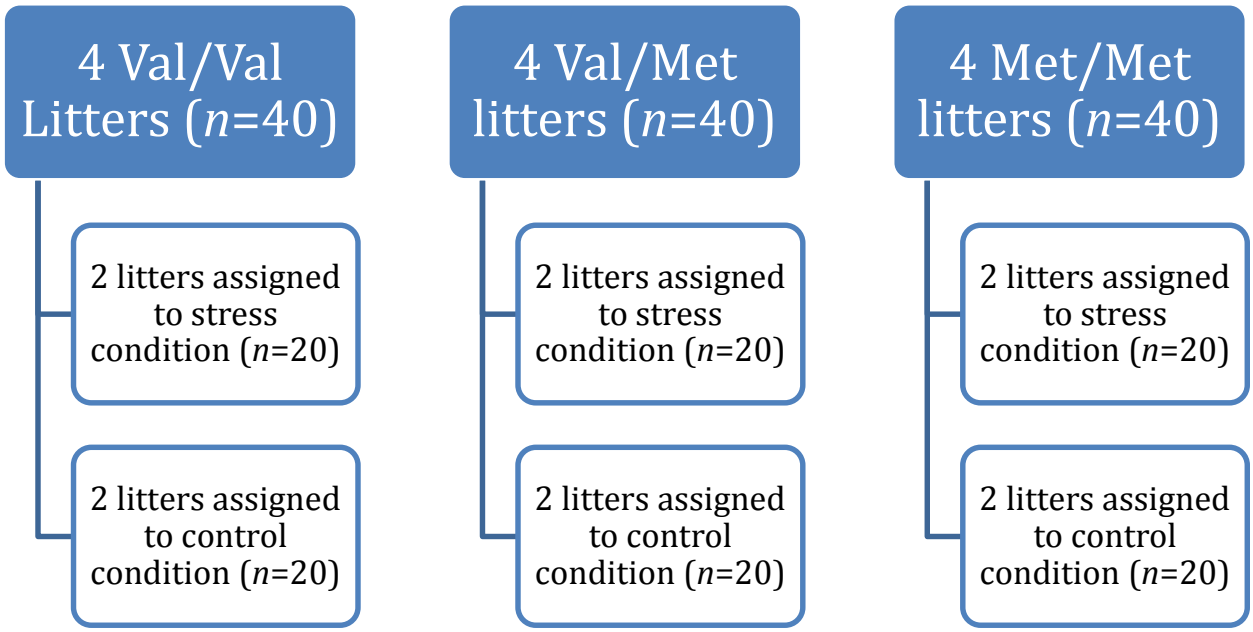

Figure 3. Assignment of *COMT* gene variant rat pups to stress or no stress condition. Half of all litters are randomly assigned to undergo the stress paradigm and the other half are assigned to the control condition.

Maternal Separation Stress Paradigm

Following the stress paradigm implemented by Hancock and Grant (2009), rats designated to the stress condition will be separated from the mother for 3 hours per day on PND 1-14, from 10:00 AM to 1:00 PM and rats in the no-stress, control condition will be handled such that they are separated from their mothers for 15 minutes per day on PND 1-14, from 9:45 AM to 10:00 AM. The control condition ensures that any effects seen are due to the stressful experience of being separated from the mother, and not from the combined stressful experiences of being

separated from the mother and being handled by experimenters. Following the handling or separation paradigm, each litter of pups will be returned to its birth cage and the mother will be returned to the birth cage as well. Litters will be left undisturbed from PND 15 through 20, and pups will be weaned on PND 21 (Hancock and Grant, 2009). Following weaning, pups will be housed in groups of three same sex and same genotype littermates in clear polycarbonate holding cages (45 cm X 24 cm X 20 cm) with open access to food and water. On PND 32, rats will be individually housed in order to implement the sucrose consumption test for anhedonia-like behavior.

Fecal Corticosterone Analysis

On PNDs 1 and 14 (i.e. before and after the stress/control paradigms), fecal matter will be collected from each litter's holding cage for examination of corticosterone levels. Fecal corticosterone will be assessed (rather than serum corticosterone) because it is a non-invasive measure that should not induce acute stress effects (Christiansen et al., 2012). It is important that the corticosterone measure itself is not stress-inducing, as otherwise corticosterone collection itself would increase corticosterone response. By examining fecal corticosterone, a non-invasive measure, the stress response (as indexed by fecal corticosterone metabolites) truly reflects the response to the experimental manipulation only. The samples will be analyzed for immunoreactive fecal corticosterone metabolites (FCM) using an a 5a-pregnane- 3b,11b,21-triol-20-one enzyme immunoassay (EIA) (Christiansen et al., 2012; Touma et al., 2003). This EIA method has been validated for use in rats (Lepschy et al., 2007). Fecal samples will be homogenized and samples of 0.25 g will be extracted with 5 ml of 80% methanol, and will be analyzed using thin layer chromatography on anti-rabbit-IgG-coated microtiter plates (Christiansen et al., 2012).

Sucrose Consumption Test

On PND 32, rats will undergo the sucrose consumption test, which is a behavioral measure of depression in the form of anhedonia-like behavior. A two bottle, overnight sucrose preference test will be given to all of the rats. Rats will be presented with a 1% sucrose solution bottle and a water bottle at 2:00 PM and the bottles will be removed the following morning at 8:00 AM (Remus et al., 2015). Sucrose solution and water levels will be recorded before and after the test, and a difference score will be recorded. Decreased sucrose consumption is associated with anhedonia-like behavior in rodents (Remus et al., 2015).

Tissue Collection and Dopamine Analysis via High Performance Liquid Chromatography (HPLC)

Tissue collection and dopamine analysis will follow the methods used by Schendzielorz et al., (2013). On PND 33, rats will be anesthetized and their brains will be perfused with iced saline before decapitation and removal of the brain. Then brain tissue will be weighed, dissected and placed in plastic microfuge tubes, and frozen on dry ice and stored at -80°C. Following the dissection methods of Schendzielorz et al., (2013), the brains will be dissected on an ice-cold glass plate. Both sides of the prefrontal cortex (PFC) will be cut from the front of the brain with a razor blade and discarded, and then a coronal cut of the brain will be made at the optic chiasm (-0.3mm). The hypothalamus will be extricated by "cutting its borders" (Schendzielorz et al., 2013) and it will be discarded. Finally, the striatum will be removed frontally from the cut based on its appearance with forceps (Schendzielorz et al., 2013). Each part will be snap-frozen in liquid nitrogen and stored at -80°C until analysis. Only the extricated striatum samples will be analyzed for dopamine concentration via high performance liquid chromatography (HPLC).

Striatal levels of dopamine will be assessed using HPLC with electrochemical detection. Striatum samples will be homogenized 0.5 ml of homogenization solution (6 parts of 0.2 M $HClO_4$ and 1 part of antioxidant solution containing oxalic acid combined with acetic acid and L-cysteine). The homogenates will then be centrifuged at 20,800 *g* for 35 minutes at 4°C, and the supernatants will be placed in 9.5 ml filter concentrators and centrifuged at 8,600 *g* at 4°C for 35 minutes (Schendzielorz et al., 2013). Following tissue collection and homogenization, high performance liquid chromatography will be used in order to assess striatal dopamine levels. The column (Spherisorb ODS2 3uM, 4.6 X 100 $mm^{2;}$ Waters, Milford, MA, USA) will be heated to 50°C. The mobile phase will consist of a solvent of 0.1 M NaH_2PO_4 buffer, 350 mg/l of octane sulfonic acid, methanol (3-6.5%), and 450 mg/l EDTA, with a pH set to 2.7. The pump (ESA Model 582Solvent Delivery Module; ESA, Chelmsford, MA, USA) flow rate will be set to 1 ml/min. Sixty µl of the filtrate will be injected into the chromatographic system with a CMA/200 autoinjector (CMS, Stockholm, Sweden) (Schendzielorz et al., 2013).

Dopamine will be detected with the ESA CoulArray Electrode Array Detector, with voltages gradually increasing from +100 to +300 mV (Schendzielorz et al., 2013). Chromatograms will be processed and dopamine concentrations will be calculated using CoulArray for windows software, with dopamine expressed as nanograms per milligram (ng/mg) of wet weight tissue. According to Schendzielorz et al., 2013, the CoulArray detector is suitable for detection and analysis of all reducible monoamines, and thus will be able to detect the presence and amount of dopamine molecules as it leaves the column.

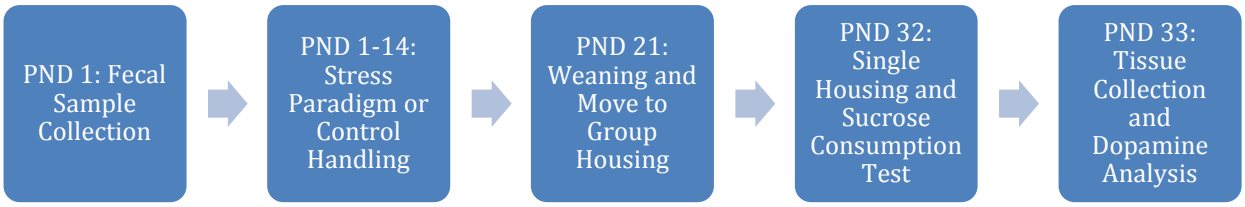

*Figure 2***.** Timeline displaying experimental paradigm.

Research Design

The present study will examine the effects of stress on a neural correlate of depression and anhedonia-like behavior across *COMT* genotype in a 2 (stress vs no stress) x 3 (Met/Val vs Met/Met vs Val/Val) design. Data will be analyzed using IBM SPSS Statistics Premium.

Preliminary analyses. As a manipulation check, I will examine fecal corticosterone metabolite (FCM) levels (nanograms per gram of dried fecal mass of individual sample) before and following completion of the stress paradigm for rats of each *COMT* genotype. I expect that across *COMT* genotype, the stress paradigm will in fact produce a stress response, as indicated by increased corticosterone, which will be tested by one paired samples t-test and two unpaired samples t-tests as a manipulation check to test for differences in FCM between stressed versus nonstressed rats on PND 1 (before the intervention) and again on PND 14 (after the intervention), as well as testing for differences in FCM within stressed rats on PND 1 versus PND 14. For these three analyses, the independent variables are date of testing (pre-stress

paradigm versus post stress paradigm), and the dependent variable is FCM.These preliminary tests serve to confirm that the stress versus control manipulations indeed had the effect they were intended to produce.

Main analyses. For the main analyses, the independent variables are stress condition (stress versus control) and *COMT* genotype (wild type Val/Val, heterozygous for variant Val/Met, homozygous for variant Met/Met). The behavioral dependent variable is the amount of sucrose solution consumed (mL) and the neurological dependent variable is the striatal dopamine concentration (nanograms per milligram [ng/mg] of wet weight tissue). I will conduct a 2 (stress vs. control) x 3 (Val/Val vs. Val/Met vs. Met/Met) ANOVA in order to assess the effects of stress condition and genotype on sucrose consumption. I will conduct a separate 2 (stress vs. control) x 3 (Val/Val vs. Val/Met vs. Met/Met) ANOVA in order to assess the effects of stress condition and genotype on striatal dopamine concentration (ng/g of wet weight tissue). Finally, I will conduct a correlation analysis in order to examine the relationship between striatal dopamine levels and sucrose consumption (anhedonia-like behavior).

Chapter 3: Proposed Results

Preliminary Analyses: Fecal Corticosterone Metabolite Levels

I hypothesize that fecal corticosterone metabolite (FCM) levels will be the same for rats in the stress condition and rats in the control condition before commencement of the stress paradigm. I expect that an unpaired t-test of FCM in stressed and control rats on PND 1 will demonstrate that no difference in FCM exists between groups before experimental manipulation (see Figure 4). In addition, I expect that an unpaired t-test will demonstrate that FCM levels differ between stressed rats and control rats at the end of the paradigm, on PND 14 (see Figure 5). In other words, I expect to find evidence from this t-test showing that the stress paradigm as compared to the control condition produces differing levels of FCM in rats in these respective conditions. Finally, I expect that a paired samples t-test comparing rats in the stress condition on PND 1 versus PND 14 will show that stressed rats differ in FCM pre- and post- stress paradigm, such that the stressed rats show increased levels of FCM at the end of the stress paradigm than at the beginning of the stress paradigm (see Figure 6).

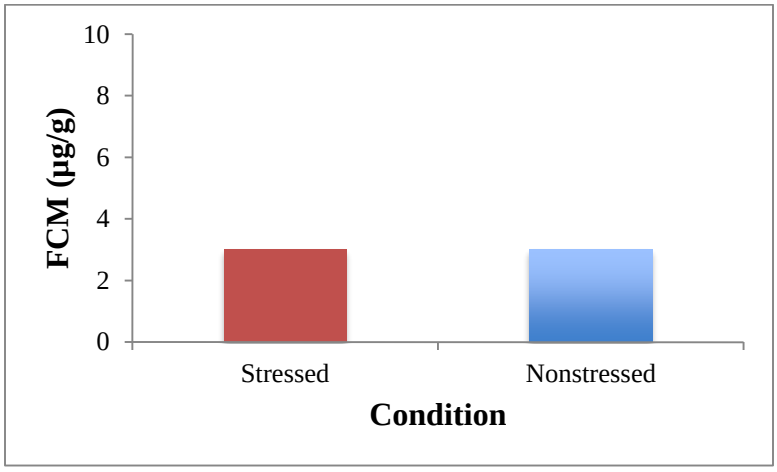

Figure 4. Results of unpaired t-test demonstrate that on PND 1 before the beginning of the stress or control paradigms, there is no difference in fecal corticosterone metabolite (FCM) across groups.

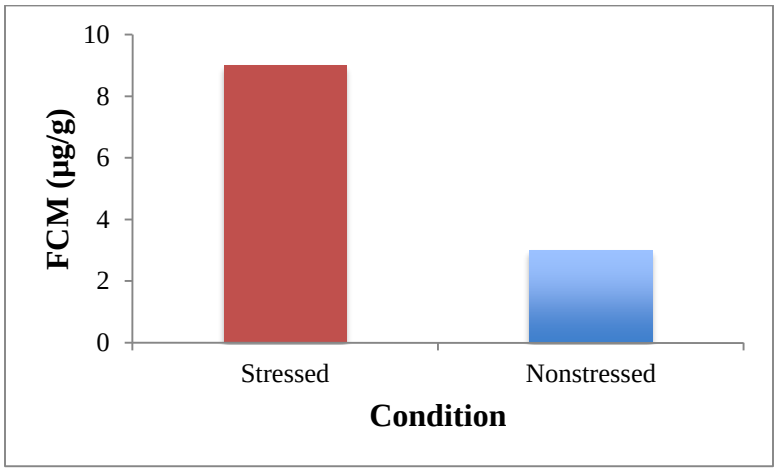

Figure 5. Results of unpaired t-test demonstrate that on PND 14, following completion of the stress or control paradigms, stressed rats have significantly increased fecal corticosterone metabolite (FCM) as compared to nonstressed rats.

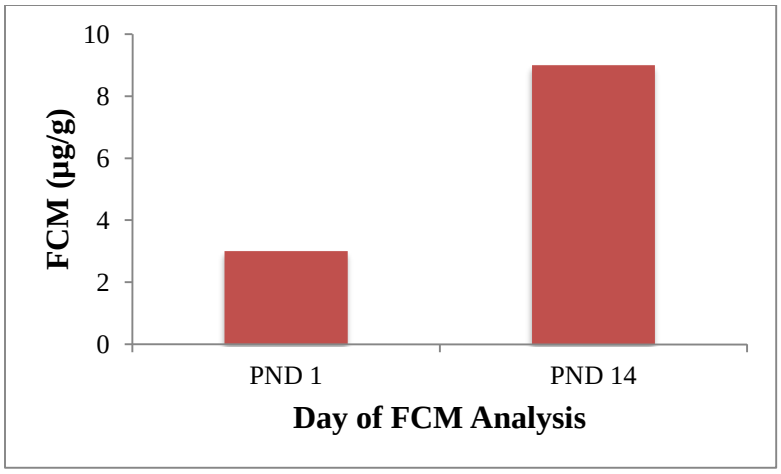

Figure 6. Results of paired t-test demonstrate that within stressed rats, fecal corticosterone metabolite (FCM) is significantly higher on PND 14, following completion of the stress paradigm, as compared to PND 1, before beginning the stress paradigm.

Anhedonia

I expect a 2 (stress vs. control) x 3 (Val/Val vs. Val/Met vs. Met/Met) ANOVA to reveal a main effect of stress, such that stressed rats consumed less sucrose solution as compared to nonstressed rats, and a stress x genotype interaction on sucrose consumption (anhedonia-like behavior), such that the effects of stress on sucrose consumption depend on genotype, as demonstrated in Figure 7. I do not expect to find a main effect of genotype. I expect planned comparisons to reveal that among stressed rats, Met/Met rats consumed the least amount of sucrose solution, displaying the most anhedonic behavior, Val/Val rats consumed the greatest amount of sucrose solution, and Val/Met rats consumed an intermediate amount of sucrose solution. Conversely, among nonstressed rats, I expect that Met/Met rats consumed the greatest amoung of sucrose solution, Val/Val rats consumed the least amount of sucrose solution, and Val/Met rats consumed an intermediate amount of sucrose solution. These results suggest that stress impacted rats differentially based on genotype, leading to differences in sucrose consumption among stressed versus nonstressed rats across genotype.

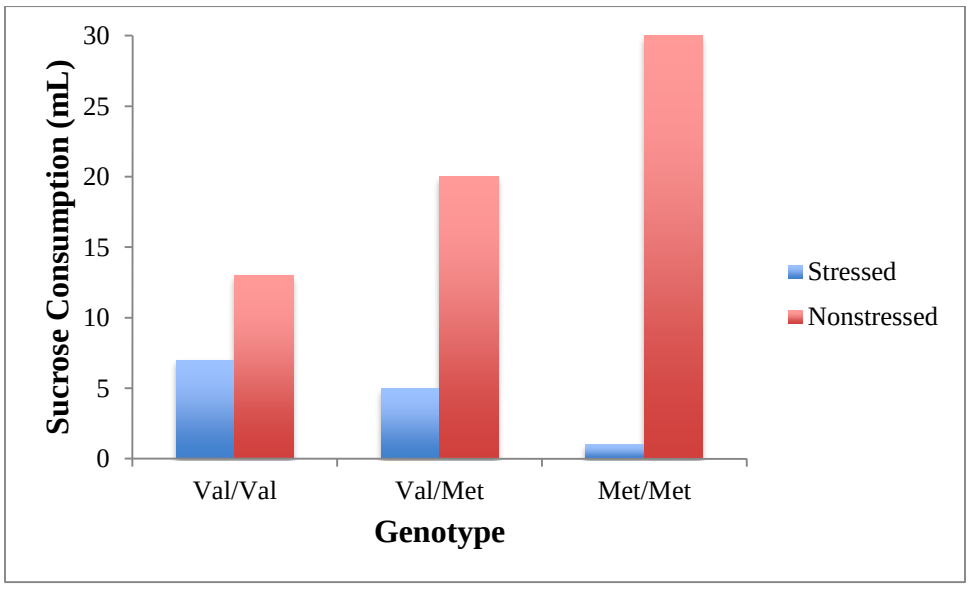

Figure 7. ANOVA results demonstrating the effects of stress and genotype on sucrose consumption. Decreased sucrose consumption indicates anhedonia-like behavior, a central tenet

of depression. Results demonstrate a main effect of stress and a stress x genotype interaction, such that stressed Met/Met rats consumed the lowest amount of sucrose solution, displaying greatest anhedonia-like behavior.

Striatal Dopamine Concentration

Similar to the behavioral results, I expect a 2 (stress vs. control) x 3 (Val/Val vs. Val/Met vs. Met/Met) ANOVA to reveal a main effect of stress, such that stressed rats display lower striatal dopamine concentrations as compared to nonstressed rats, and a stress x genotype interaction on striatal dopamine concentration, such that the effects of stress striatal dopamine concentration depend on genotype, as depicted in Figure 8. I do not expect to find a main effect of *COMT* genotype. I expect planned comparisons to reveal that among stressed rats, Met/Met rats demonstrated the lowest striatal dopamine concentrations, Val/Val rats demonstrated the highest striatal dopamine concentrations, and Val/Met rats displayed intermediate striatal dopamine concentrations. Conversely, among nonstressed rats, I expect that Met/Met rats displayed the highest striatal dopamine concentrations, Val/Val rats displayed the lowest striatal dopamine concentrations, and Val/Met rats displayed intermediate striatal dopamine concentrations. These results suggest that stress impacted rats differentially based on genotype, leading to differences in striatal dopamine concentrations among stressed versus nonstressed rats across genotype.

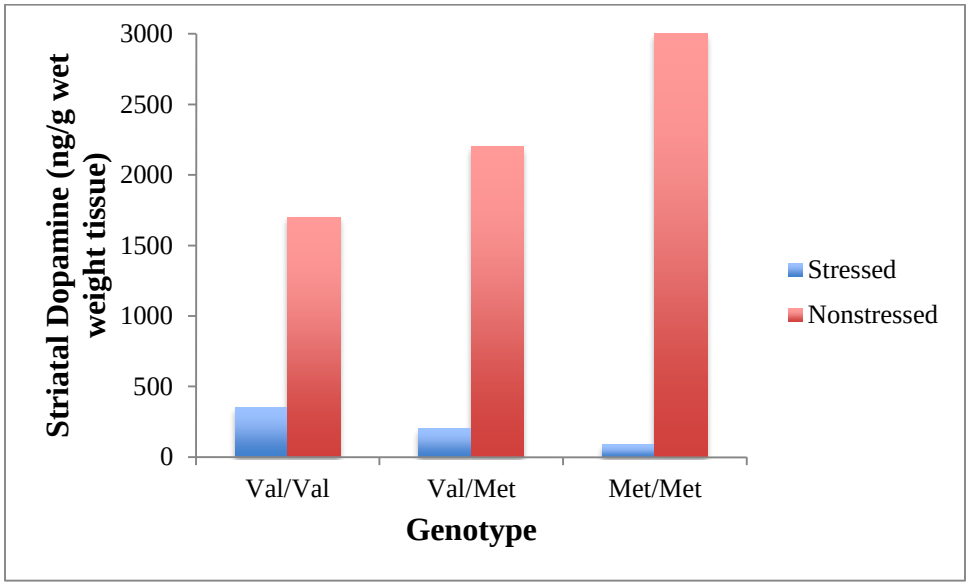

Figure 8. ANOVA results demonstrating the effects of stress and genotype on dopamine concentration in rat striatum. Results demonstrate a main effect of stress and a stress x genotype interaction, such that stressed Met/Met rats display the lowest total prefrontal and striatal dopamine concentration.

Correlation

I expect to find a positive correlation between sucrose consumption and dopamine levels, such that increased dopamine is associated with increased sucrose consumption (decreased anhedonia), as demonstrated in Figure 9. This result would provide some evidence that decreased dopamine is associated with anhedonia-like behavior, which can be considered an endophenotype of depression.

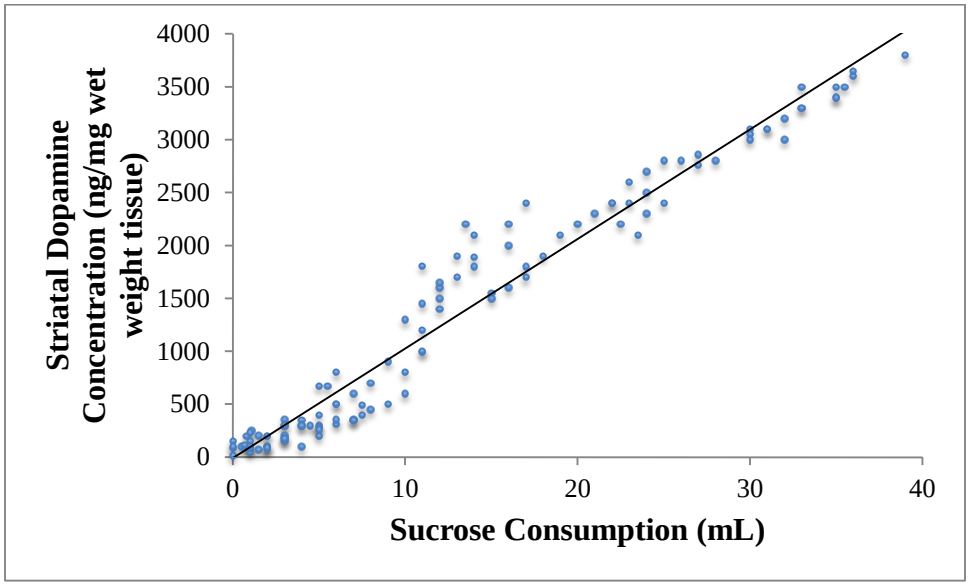

Figure 9. Sucrose consumption is positively correlated with dopamine concentration, such that higher dopamine levels are associated with increased sucrose consumption. Decreased dopamine is associated with decreased sucrose consumption, which is indicative of anhedonia.

Chapter 4: Discussion

The aim of the present study was to investigate the role of one gene x environment interaction in depression. Specifically, the present study aimed to examine the effects of stress on striatal dopamine concentrations and anhedonia as they relate to depression via the *COMT* Val^{158}Met polymorphism. I compared the effects of stress manipulation versus control handling on anhedonia and striatal dopamine concentration across different *COMT* genotypes (Val/Val, Val/Met, Met/Met) in a rodent model. I hypothesized that stressed Met/Met *COMT* genotype rats would demonstrate the lowest striatal dopamine concentrations and highest levels of anhedonia-like behavior, and thus the greatest vulnerability to depression, based off of recent findings from human association studies (Mandelli et al., 2006; Opmeer et al., 2010; Antypa et al., 2013). I also hypothesized that striatal dopamine concentration would be positively correlated with sucrose consumption, such that higher dopamine levels are associated with increased sucrose consumption, and decreased dopamine is associated with decreased sucrose consumption, or anhedonia-like behavior. Taken together, the proposed behavioral, neurological, and correlational results support these hypotheses.

The proposed behavioral results clarify and resolve conflicting and contradictory evidence (Antypa et al., 2013; Lachman et al., 1996; Opmeer et al., 2010; Mandelli et al., 2006) on which *COMT* genotype confers the greatest vulnerability to depression by indicating that the effects of stress depend on *COMT* genotype to predict sucrose consumption/anhedonia-like behavior. Results of the sucrose consumption test revealed that there was a main effect of stress and a genotype x stress interaction. In terms of the main effect of stress, collapsing across genotype, nonstressed rats consumed significantly more sucrose solution, as compared to stressed rats. In other words, the effects of stress triggered anhedonia-like behavior. These

findings are consistent with previous findings demonstrating that stress leads to anhedonia (Bogdan and Pizzagalli, 2006; Enkel et al., 2010).

The interaction results suggest that the nonstressed Met/Met rats consumed the greatest amount of sucrose solution, displaying the lowest anhedonia-like behavior, while the stressed Met/Met rats consumed the lowest amount of sucrose solution, displaying the greatest anhedonia-like behavior. In other words, in the absence of stress, the Met/Met genotype seems to be protective against anhedonia, but in the presence of stress, the Met/Met genotype confers significant vulnerability to anhedonia. These behavioral results are consistent with previous research demonstrating that the Met allele is more reactive to stressors (Opmeer et al., 2010, Mandelli et al., 2006), and thus the "the met-allele genotype could enhance predisposition to MDD by altering the reactivity to stressors," (Opmeer et al., 2010). For example, a recent study demonstrated that Met/Met participants exhibit heightened cortisol reactivity and impaired recovery after a stressful task (Alexander et al., 2011). Therefore, these results are in accordance with previous research that shows that the Met/Met genotype interacts uniquely with stress to lead to enhanced vulnerability to depression.

The proposed neurological results further clarify and resolve conflicting and contradictory evidence (Antypa et al., 2013; Lachman et al., 1996; Opmeer et al., 2010; Mandelli et al., 2006) on which *COMT* genotype confers the greatest vulnerability to depression by indicating that the effects of stress depend on *COMT* genotype to predict striatal dopamine concentration (see Figure 10). Results concerning striatal dopamine analysis via HPLC revealed that there was a main effect of stress and a genotype x stress interaction. In terms of the main effect of stress, collapsing across genotype, nonstressed rats exhibited significantly greater striatal dopamine concentrations, as compared to stressed rats. This finding is in accordance with

findings that suggest that both chronic and acute stress lead to decreased striatal dopamine concentrations (Hu et al., 2014).

The interaction results suggest that overall, the nonstressed Met/Met rats exhibited the greatest striatal dopamine concentrations, while the stressed Met/Met rats exhibited the lowest striatal dopamine concentrations. Similar to the behavioral results, this genotype x stress interaction indicates that in the absence of stress, the Met/Met genotype seems to be protective against depression, but in the presence of stress, the Met/Met genotype confers significant vulnerability to depression, via dopaminergic processes. Again, these neurological results are consistent with previous research demonstrating that the Met allele is more reactive to stressors (Opmeer et al., 2010, Mandelli et al., 2006; Walder et al., 2010), and are in accordance with evidence that the dopaminergic system is sensitive to stress (Pani et al., 2000) and, specifically, that dopamine concentration in the striatum is sensitive to stress (Hu et al., 2014). One way stress could enhance vulnerability to depression is through the influence of stress on the more highly stress-reactive dopaminergic system in individuals with the met allele (Opmeer et al., 2010; Alexander et al., 2011). It is possible that stress causes upregulation of COMT in met allele carriers, leading to increased dopamine catabolism and decreased striatal dopamine concentrations; however future research is necessary in order to examine COMT activity directly in regards to this question.

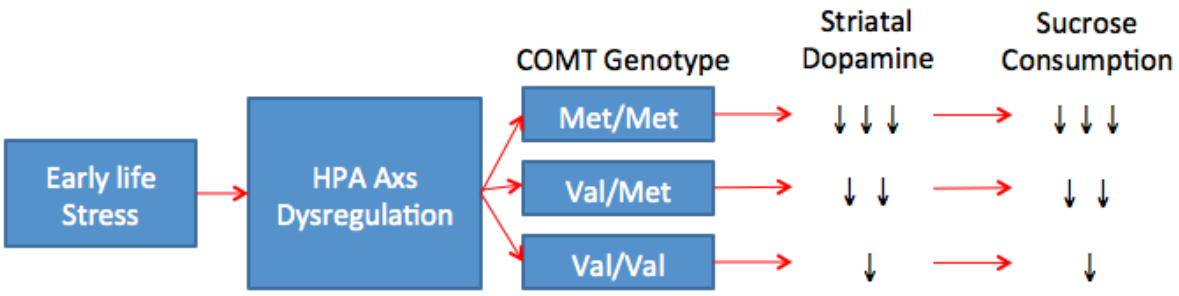

Figure 10. Hypothesized mechanistic relationship underlying stress x genotype interaction. Stress leads to HPA dysregulation and potentially *COMT* upregulation to result in decreased striatal dopamine concentration and decreased sucrose consumption (anhedonia), with the greatest reductions in striatal dopamine and sucrose consumption among those with the Met/Met genotype.

The proposed correlational results indicate that sucrose consumption and striatal dopamine concentration are positively correlated, such that increased dopamine is associated with increased sucrose consumption (decreased anhedonia), and decreased dopamine is associated with decreased sucrose consumption (anhedonia). These proposed results provide some evidence that decreased dopamine is associated with anhedonia-like behavior, which can be considered an endophenotype of depression (Antypa et al., 2013). Furthermore, these results support the findings that dopamine function underlies processes associated with anhedonia (Argyropoulos and Nutt, 2013).

One limitation of the proposed study is that random assignment to stress condition was done by litter instead of completely randomly- by individual rat pup. This method of random assignment leaves open the possibility that extreme between litter variance could interfere with

the results (between group differences). However, random assignment by litter was necessary for the present study in order to perform the maternal separation paradigm as outlined by Hancock and Grant (2009). In this paradigm, following daily periods of separation, litters were placed back with their mothers, as the stress paradigm begins when the rats are one day old and need to be with the mothers in order to feed. Future research might implement different types of stress paradigms focused on later periods of development, in which rats can individually be randomly assigned to stress condition.

Future research might also further investigate mechanistic pathways implicated in depression with regards to dopamine, as the present study did not investigate the specific dopamine pathways or mechanistic functions through which stress might modulate COMT activity in regards to specific *COMT* genotypes. For example, one proposed dopamine pathway involved in depression concerns mesolimbic dopaminergic projections from the ventral tegmental area to the nucleus accumbens and the medial prefrontal cortex (Antypa et al., 2013). This pathway is said to play a role in learning and reward (Antypa, 2013), and thus dysregulation in this pathway may play a role in anhedonia and reduction in motivation, common symptoms of depression (Nestler and Carlezon, 2006).

Other proposed pathways that may be implicated in depression are the limbic-cortical-striatal-pallidal-thalamic circuit (LCSPT) (Hamon and Blier, 2013; Drevets et al., 2008) and a prefrontal-limbic network that is modulated by the hypothalamus, basal ganglia, and midbrain has also been proposed (Bennett, 2011). The LCSPT pathway connects the orbital prefrontal cortex, amygdala, hippocampus, ventromedial striatum, mediodorsal and midline thalamic nuclei, and ventral pallidum (Hamon and Blier, 2013; Drevets et al., 2008). This system has been implicated in fear, anxiety, self-reference, visceral response, and reward (Hamon and Blier,

2013; Price and Drevets, 2012). The prefrontal limbic network includes the anterior cingulate cortex, amygdala, and hippocampus as part of an interconnected prefrontal and limbic network that is dysregulated in depression (Bennett, 2011). It remains to be investigated whether upregulation of COMT in Met/Met individuals in response to stress may impact dopamine concentrations and signaling via these pathways, influencing anhedonia.

Furthermore, it is possible that gene x gene interactions exist that would further elucidate the relationship between stress, COMT, and depression (Antypa et al., 2013). In such an instance, multiple polymorphic variations might interact to increase vulnerability to depression. For example, Jabbi et al., (2007) demonstrated an interaction between polymorphic variations in genes coding for COMT and monoamine oxidase A (MAOA). This COMT by MAOA interaction influences HPA axis activity, as indexed by plasma adrenocorticotropin hormone (ACTH) stress response, in response to acute stress (Jabbi et al., 2007). This study provides evidence that the *COMT* met allele genotype interacts with the low activity *MAOA* genotype in order to alter the stress response. Gene-gene interactions might further explain heterogeneity in depression and contribute to our understanding of the diverse etiology of depression. Future research should examine potential gene-gene interactions in depression, and specifically concerning *COMT* gene variants.

Finally, the present study does not examine the potential role of epigenetics in modulation of *COMT* in conferring vulnerability to depression. Epigenetic changes in gene expression occur through the activation of chromatin, by acetylation and methylation of histones (Porcelli et al., 2011). Gene expression via epigenetic factors is controlled either by chemically altering the DNA (adding a methyl group, or methylation) or when histones, the proteins that pack DNA into chromatin, are modified (Qiu, 2006). Histones determine whether, "the

chromatin is tightly packed, in which case gene expression is shut down (or silenced), or relaxed, in which case gene expression is active" (Qiu, 2006). Future research should investigate the potential that early life stress combined with the Met allele variant of *COMT* may increase vulnerability to depression via epigenetic modification of COMT and subsequent changes to dopamine catabolism and activity in prefrontal and limbic areas.

Taken together, the proposed results of the present experiment contribute to and clarify our understanding of one mechanistic route to depression, possibly explaining some of the heterogeneity in the etiology of depression. These results suggest that effects of early life stress on anhedonia and striatal dopamine concentrations depends on variation in *COMT*, such that stress interacts with the Met/Met genotype to confer greatest vulnerability to depression. This is seen in stressed Met/Met rats, which are expected to exhibit the lowest sucrose solution consumption (greatest anhedonia-like behavior) and the lowest striatal dopamine concentrations. The results of the correlation analysis also provide support for the notion that sucrose consumption and dopamine concentration are positively related, and diminished dopamine levels may be implicated in anhedonia. Overall, these results highlight the role of dopamine and COMT in depression, suggesting that stress interacts with *COMT* gene variants to modulate dopamine activity, conferring vulnerability to depression.

These hypothesized results may have significant implications in terms of prevention and treatment of depression in individuals with one or more copies of the Met allele, as these results indicate that individuals with the Met allele, following early life trauma, are at an increased vulnerability to developing depression. Further research is warranted in order to investigate mechanistic pathways involved in the relationship between stress, the functional *COMT* Val^{158}Met polymorphism, and depression, and in order to research potential forms of

psychotherapeutic treatment aimed at downregulating COMT for instance, or increasing extracellular dopamine by other mechanisms.

As "anhedonia may serve as an important prognostic tool for identifying youth who may be at risk for a poorer or delayed recovery" (McMakin et al., 2012), anhedonia can be considered as an endophenotype of depression that should be treated in a different manner than just SSRIs because it is related to dopamine dysfunction (Nestler and Carlezon, 2006). Because anhedonia has been shown to predict poorer recovery among adolescents with SSRI treatment-resistant depression (McMakin et al., 2012; Uher et al., 2012), it may be beneficial to investigate the anhedonia and the functional *COMT* Val^{158}Met polymorphism in relation to treatment outcomes. As the present proposed results suggest that early life trauma, and presence of the *COMT* Val158Met polymorphism predict increased anhedonia and decreased dopamine concentrations, it is possible that the this polymorphism predicts worse treatment outcomes with SSRI antidepressants. In other words, these hypothesized findings suggest that dopamine dysfunction, *COMT* Met/Met genotype, and early life stress not only increase vulnerability to depression and increased depression symptoms, but that this "type" of depression may warrant a different type of treatment, with drugs that target dopamine. Future research should investigate treatment response to a variety of antidepressant treatments in a rodent study or in a human study with *COMT* Val158Met polymorphism, depression, and early life stress.

Conclusion

The proposed behavioral results clarify and resolve conflicting and contradictory evidence (Antypa et al., 2013; Lachman et al., 1996; Opmeer et al., 2010; Mandelli et al., 2006) on which *COMT* genotype confers the greatest vulnerability to depression by indicating that the effects of stress depend on *COMT* genotype to predict sucrose consumption/anhedonia-like

behavior. Although any animal model is an imperfect representation of the human condition and should be interpreted with caution, the hypothesized results of the proposed experiment shed light on the underlying mechanisms of depression induced by early life trauma. Specifically, the proposed results suggest that early life stress leads to HPA axis dysregulation that is modulated by *COMT* genotype to influence relative decreases in striatal dopamine concentration and sucrose consumption (see Figure 10). The hypothesized results suggest that the negative effects of early life stress are most pronounced for the *COMT* Met/Met genotype, which shows the greatest decreases in striatal dopamine concentration and sucrose consumption, as compared to the Val/Met and Val/Val genotypes. The negative effects of early life stress on striatal dopamine concentration and sucrose consumption are least pronounced for the Val/Val genotype. Taken together, the proposed results suggest that the interaction between early life trauma and the *COMT* Met/Met genotype confers the greatest vulnerability to developing depression with anhedonia symptoms.

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Appendix A

Proposal for Submission to the Institutional Animal Care and Use Committee (IACUC)

i. Statement of Purpose

The proposed study aims to test the interaction between early life stress and *COMT* genotype on striatal dopamine concentration and the development of anhedonia in a rat model of depression. The present study aims to clarify and resolve conflicting and contradictory evidence (Antypa et al., 2013; Lachman et al., 1996; Opmeer et al., 2010; Mandelli et al., 2006) on which *COMT* genotype confers the greatest vulnerability to depression.

ii. Benefit

Results of the proposed experiment will allow for better understanding of one gene-environment interaction as it relates to the development of depression. These results could have implications for treatment of depression in individuals at greater risk for developing the disorder.

iii. Why Rats? (not a lower phylogenic class..)

Genetic manipulation of rats allows for the isolation of specific genetic variants in an otherwise isogenic background. Complex phenotypes like depression can be studied in rats, using validated behavioral tests probing specific components of depression, such as anhedonia, that map onto human symptoms. These outcome measures cannot be studied in lower phylogenetic classes.

iv. Ethical treatment in Animal Procedures

During the maternal separation paradigm, rat pups may experience a sustained period of psychological distress, but this distress will be relieved when the pups are returned to their mothers. This distress is necessary in order to model early life trauma in humans. In all other procedures rats will experience no pain or distress or momentary pain or distress. Tail snipping

procedures for genotyping the pups will follow established ethical care standards (TaqMan genotyping assay, Applied Biosystems, Irvine, CA) and rats will be anesthetized prior to sacrifice and brain dissection.

Estimated Budget

Expenditure	Description/Notes	Cost
Genetically manipulated rat dams	Purchased from Charles River Genetically Engineered Model Services	$500 setup per genetic manipulation, plus $150 per pregnant dam
Rat housing fees	Cages, lining, food, water bottles, incubators, sucrose solution,	$4.41 cage setup, plus $1.47 per cage per day for housing
Behavioral Testing Materials	Testing chamber, sucrose solution	$3,200
Chemicals and PCR and HPLC equipment	HPLC columns, reagents, buffers, vials	$5,000
Payment of Research Assistants	Two laboratory technicians, 40 hours/week, 10 weeks	$14,800
Miscellaneous laboratory supplies	Pipette tips, dissection materials, etc,	$7,000

Experiment Timeline

Procedure	Duration	Study Milestone
Housing pregnant mothers until birth	1 week	Week 1: Litter delivery; culling
Experimental Paradigm PND 0- PND 33	~5 weeks	Week 6: Early life stress, maternal separation vs. control handling; collection of fecal material; sucrose consumption measurements
Brain Dissection	1 week	Week 7: Collection of striata for analysis of dopamine by HPLC
Data Analysis	3 weeks	Week 10: Completion of study