The role of serotonergic dysfunction in the etiology of affective disorder in a Huntington's disease translational mouse model

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ABSTRACT OF THE DISSERTATION

The role of serotonergic dysfunction in the etiology of affective disorder in a Huntington's disease translational mouse model

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Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder that manifests with a triad of psychiatric, cognitive, and motor symptoms. The pathophysiology of the psychiatric symptoms is not well understood but is frequently associated with serotonergic dysfunction in limbic-related circuits in the brain. However, it remains unknown how the serotonergic system is impaired in HD. The primary aim of this thesis is to assess the use of a translational mouse model of HD to better understand the contribution of serotonergic dysfunction in the etiology of affective disorders in the disease; and to assess the use of novel therapeutics for the amelioration of symptoms. I conducted tests to quantify the incidence of affective disorder in mice and observed that the BACHD mouse model displays anxiety-like and depressive-like behaviors, which are the two most common psychiatric symptoms in HD patients. Importantly, I found evidence that the psychiatric symptoms in the BACHD mice develop prior to the onset of motor deficits, similar to HD patients. The similar behavioral phenotype of this mouse model enabled further investigation into the role of serotonergic neurotransmission in HD. With the use of in vivo microdialysis, I quantified serotonergic efflux, which is a product of serotonin release, reuptake, metabolism and receptor binding. Serotonin efflux was reduced in the ventral

hippocampus but not the dorsal striatum, suggesting that serotonin efflux is only reduced in the limbic ventral hippocampus and that monoaminergic activity (including dopamine) in the dorsal striatum remains intact. With additional tests, I determined that activation of the 5-HT1A receptor using an agonist has an anxiolytic and potential antidepressant effect in the BACHD mouse model. Acute administration of the selective serotonin reuptake inhibitor ameliorated depressive symptoms in the BACHD mice. The dual antidepressant and anxiolytic effect of activation of the 5-HT1A receptor is suggestive that serotonergic dysfunction in the BACHD mice is a result of impaired communication in postsynaptic targets of the serotonergic system.

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5-HT	Serotonin
aCSF	Artificial Cerebrospinal Fluid
BAC	Bacteria Artificial Chromosome
CAG	Polyglutamine Repeat
CSF	Cerebrospinal Fluid
DA	Dopamine
dHPC	Dorsal Hippocampus
DRN	Dorsal Raphe Nucleus
DSM	Diagnostic and Statistical Manual of Mental Disorders
EPM	Elevated Plus Maze
EZM	Elevated Zero Maze
FST	Forced Swim Test
HD	Huntington's Disease
HTT	Huntingtin Protein
IT	Interesting Transcript
MAO	Monoamine Oxidase
MAOI	Monoamine Oxidase Inhibitor
mHTT	Mutant Huntingtin Protein

mPFC	Medial Prefrontal Cortex
MRN	Median Raphe Nucleus
MSN	Medium Spiny Neurons
OF	Open Field
PFC	Prefrontal Cortex
PolyQ	Polyglutamine
SERT	Serotonin Transporter
SSRI	Selective Serotonin Reuptake Inhibitor
TST	Tail Suspension Test
UHDRS	Unified Huntington's disease Rating Scale
WT	Wildtype
WT-HTT	Wildtype Huntingtin Protein
vHPC	Ventral Hippocampus
YAC	Yeast Artificial Chromosome

CHAPTER I

INTRODUCTION

1.1 Background and Significance

Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder that manifests in middle age with cognitive, psychiatric and motor symptoms. Importantly, the cognitive and psychiatric symptoms emerge prior to the motor symptoms and thus require treatment much earlier to the onset of chorea, the hallmark diagnostic marker of HD. This thesis will focus particularly on the psychiatric symptoms of HD.

According to Rowe et al. (2012) selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed medication for psychiatric symptoms exhibited by HD patients. SSRIs are commonly prescribed to HD patients because this class of drugs is considered the most effective treatment for psychiatric symptoms in otherwise healthy individuals. This was reported in a few case and clinical studies that investigated the efficacy of SSRIs in treating the affective symptoms of HD (Andersen and Marder 2001, Beglinger et al. 2013). However, case studies often do not control for disease severity, disease history, or other factors that could confound results of drug efficacy. Therefore, additional clinical studies are needed to address remaining questions on the efficacy of SSRIs as the most effective treatment for psychiatric symptoms in HD patients.

Whilst it is necessary for additional clinical studies to be conducted to identify beneficial treatments for these symptoms in patients, there is also a need and benefit to studying the efficacy of pharmacological treatment for affective abnormalities in a translational mouse model of HD. The pharmacological improvement of symptoms provides insight into the underlying etiology of the anxious and depressive symptoms in HD and can also provide insight into the pathophysiology of anxiety and depression in

non-disease states, as the symptoms manifest *de novo* in the animal models. This is of interest given that other mouse models of psychiatric disorders experimentally induce anxious and depressive symptoms by manipulating different aspects of serotonergic signaling, which is suspected to underlie these symptoms in HD. This common feature between HD and non-HD affective disorders could be useful for understanding the neurobiology of affective disorders. Therefore, investigating the underlying neurobiology of these symptoms in HD could be informative of how impairments in the serotonin system can lead to affective disorders in healthy individuals and in comorbid neurodegenerative disorders.

The focus of this dissertation is to examine pathological changes in the serotonin system in relation to the psychiatric symptoms in HD. Furthermore, a goal of this dissertation was to examine the efficacy of commonly prescribed medications and novel treatments for the amelioration of affective symptoms in a translational mouse model of HD. SSRIs are typically used for the treatment of anxiety and depression in healthy individuals and HD patients. However, recent evidence suggests that pharmaceutical drugs targeting serotonin receptors may serve as a better treatment for affective disorders in both patient populations. The potential of serotonin receptor agonists as a novel treatment may indicate a potential mechanism underlying affective disorders.

1.2 Huntington's disease

Huntington's disease is caused by a mutation in exon 1 of the IT15 ("interesting transcript") gene, located on chromosome 4 (The Huntington's Disease Collaborative Group 1993). The coded portion of exon 1 consists of a CAG triplet repeat, which

encodes for the huntingtin (HTT) protein, a ubiquitous protein that contains a polyglutamine (polyQ) chain. In healthy individuals, the HTT protein encodes a normal repeat length of approximately10- 35 CAG repeats (Reiner et al. 2003). The length of the triplet repeat varies in individuals and the probability of developing the disease is dependent on repeat length. Individuals with a CAG repeat length of 36-39 have a slight chance of developing the disease relative to individuals with more than 40 CAG repeats, who have a 100% probability of developing the disease (Vonsattel and DiFiglia 1998). Huntington's disease can also manifest in patients with a repeat length of greater than 70, which causes a rare form of juvenile Huntington's disease that manifests in early childhood.

There are five stages of manifest Huntington's disease, in addition to two premanifest stages (Ross et al. 2014). These two premanifest stages of HD occur prior to the onset of Huntington's chorea. During the first premanifest or presymptomatic stage, patients with the genetic mutation show no symptoms. During the second premanifest stage, the prodromal stage, patients begin to exhibit non-motor symptoms including cognitive dysfunction in addition to subtle motor symptoms and psychiatric symptoms. This period of early HD can emerge 5-15 years before manifest-HD. The five manifest stages emerge around middle age (40-50 years old) with the onset of chorea, the hallmark motor symptom. Symptoms in the late stages include a worsening of motor and cognitive symptoms and an eventual total loss of motor ability and death approximately 15-20 years after disease onset (Sørensen and Fenger 1992). These stages are identified using the Unified HD Rating Scale (UHDRS), which quantifies the severity of the motor, cognitive, behavioral, emotional and functional impairment in patients. The duration of

each stage of the disease varies amongst patients ranging from the first three stages lasting 2-6 years, and the fourth and fifth stage lasting approximately ten years (Kirkwood et al. 2001).

1.2.1 Huntingtin protein

The primary pathological changes in HD include neuronal death and white matter loss, which is a result of the loss of the normal huntingtin protein function and toxic gain-of-function resulting from the abnormal protein expression (Saudou and Humbert 2016). The huntingtin protein is a 348 kDalton protein that is ubiquitous to all cells in the brain and exists in invertebrates and all mammalian species. This protein has a role in early brain development that is distinct from its role in mature organisms. This was discovered from a series of experiments that knocked out huntingtin protein expression prenatal in rodents (Duyao et al. 1995, Nasir et al. 1995, Zeitlin et al. 1995). This knockout resulted in the fatality of all pups. Furthermore, in a mouse model that was lacking only 50% of normal huntingtin protein expression; the mice survived gestation but expressed defects in cortical and striatal brain development and only survived a few days postnatal (White et al. 1997). This is suggestive of the importance of the huntingtin protein for neuronal survival in the cortex and striatum, the two main regions affected in HD patients. The role of this protein was further elucidated through studies in mature organisms. In mature mammals, the role of the protein changes and is mainly involved in vesicular trafficking, ciliogenesis, endocytosis, vesicular recycling, cell division, cellular homeostasis and endosomal trafficking (Saudou and Humbert 2016). Wild-type (normal) HTT protein is neuroprotective, particularly in striatal neurons, through the transportation of BDNF via

cortico-striatal synapses. Striatal neurons do not produce BDNF, but the release of vesicular BDNF from cortical neurons binds to TrkB receptors on striatal cells. The TrkB receptors activate second messenger pathways that regulate cell survival (Liot et al. 2013). Therefore, with the loss of the normal protein, cellular processes such as synaptic transmission and cell survival are affected. In addition to the loss of normal protein function, there is a toxic gain of function of the mutant protein (Saudou and Humbert 2016). The mutant HTT protein is cleaved into fragments containing the abnormal CAG repeats. These fragments enter the nucleus and interfere with transcription. The elongated mutant protein also forms aggregates in several brain areas, including major cortical regions and the basal ganglia, primarily in the striatum (Vonsattel 2008). The presence of the mutant protein activates autophagy pathways, which has been observed in several HD mouse models. Furthermore, the presence of protein aggregates were seen in the brain of HD patients postmortem, indicating that mutant protein expression drives the neurodegeneration observed in HD patients (Vonsattel 1985).

1.2.2 Human pathophysiology of HD

There is little overlap between the neural correlates of the motor, cognitive, and psychiatric symptoms in HD. The etiology of the motor symptoms is the best understood in humans. It is known that the decline in motor performance results from the death of medium spiny neurons (MSNs) in the striatum, the main input nucleus of the basal ganglia (Guo et al. 2012). The death of the medium spiny neurons in the striatum is classified in five stages of severity (Vonsattel and DiFiglia 1998). Approximately 40%, 50, 58%, 73% and 76% of MSNs are lost in stage 0, 1, 2, 3, and 4 respectively (Vonsattel

2008; Guo et al. 2012). Importantly, the degree of neuronal loss correlates with the severity of motor symptoms, with more severe symptoms associated with a higher percentage of neuronal loss in the striatum.

The two types of MSNs of the striatum degenerate in different phases. Dopamine-type 2 receptor-expressing MSNs (D2-MSNs) degenerate first, with the loss of dopamine-type 1 receptor-expressing (D1-MSNs) occurring at a later stage (Albin et al. 1999). The early loss of D2-MSNs results in decreased activation of the 'indirect' basal ganglia pathway. The indirect pathway is crucial for inhibiting unwanted, competing movements (Wang et al. 2015; Mink 1996). This loss of inhibition results in involuntary muscle movement. In HD, involuntary muscle control manifests as "jerk-like" movements of the head, neck, limbs and trunk that occlude the ability to initiate normal, voluntary movement such as walking. This is the hyperkinetic phase of HD and is most identifiable by the display of chorea, which translates to "dance-like" movement (Jacobs et al. 2016). Late-stage HD is characterized by hypokinesis and bradykinesia (slowing of movement), as evident by increased difficulty to initiate movement. The hypokinetic stage of HD is a result of the loss of the D1-MSNs. The D1 MSNs are part of the 'direct' basal ganglia pathway. This pathway facilitates the initiation of movement. As a result of the loss of D1 MSNs, this pathway is inhibited and the facilitation of action initiation is lost, resulting in hypokinesis and bradykinesia.

The final stages of MSN neuronal loss occurs concomitantly with tissue loss in other brain areas. Neuronal degeneration in the output nuclei of the basal ganglia, neocortex, thalamus, cerebellum and white matter loss occurs during later stages of the

disease, when the majority of MSNs are already lost (Vonsattel 2008). Whilst the loss of neurons in the basal ganglia is known to cause the motor symptoms of HD, it is hypothesized that this is unrelated to the psychiatric symptoms of HD. A study by Paulsen and colleagues (2008) assessed the degree of volume loss in symptomatic HD patients during the prodromal stage, in which patients exhibit few motor symptoms, and robust cognitive and psychiatric symptoms. Symptomatic patients exhibited cortical thinning in the anterior, parietal, occipitotemporal, and temporal lobe, anterior cingulate cortex, precuneus, white matter loss in cortical regions, as well as degeneration in the thalamus and striatum. The grey and white matter loss was significantly associated with decreased motor function and declined performance in cognitive tasks (Paulsen et al. 2001; Rosas et al. 2005; Beglinger et al. 2013; Tang and Feigin 2012). However, there was no association between striatal or cortical degeneration, or white matter loss with the psychiatric symptoms (Paulsen et al. 2008). This suggests that the psychiatric symptoms of HD are not solely related to structural changes in striatum and cortex, the main brain regions affected in HD.

1.3 Affective symptoms in human HD patients

The earliest studies of the psychiatric symptoms of HD identified the spectrum of behavioral abnormalities that encompass the affective disorders in HD. This includes irritability and aggression (Pflanz et al. 1991; Nance and Sanders 1996; Marder et al. 2000); apathy (Pflanz et al. 1991); depression (Folstein et al. 1983; Jensen et al. 1993; Shiwach 1994; Slaughter et al. 2001); anxiety (Caine and Shoulson 1983; Levy et al. 1998); and obsessive-compulsive disorder (De Marchi et al. 1998; Marder et al. 2000).

One caveat of affective abnormalities in HD patients is whether this is a reaction to diagnosis and disease onset or if the high incidence of symptoms is comorbid with the disease. Julien and colleagues (2007) conducted a study in genetic carriers and familial non-carriers of the genetic mutation in which they investigated the prevalence of affective disorders in both populations. This was done in patients naïve to pharmacological treatments for psychiatric symptoms and who were unaware of their genetic status. All individuals tested had a family history of HD but did not undergo genetic prediction testing until after being interviewed. Additionally, they selected individuals that did not exhibit any motor abnormalities, which would have been informative to the patient as to their disease status. Furthermore, the clinicians were blind to the genetic status of individuals tested, preventing bias. Affective symptoms were diagnosed and quantified using the Diagnostic and Statistical Manual of Mental Disorders (DSM-III). Genetic carriers exhibited a higher incidence of depressive and anxious behaviors including Major Depressive Disorder and Generalized Anxiety. It was also determined that carriers closer to disease onset at the time of their assessment (determined in follow-up interviews) had an even higher incidence of affective disorders compared to carriers farther from diagnosis. Thus, it is highly likely that the prevalence of affective disorders in HD is a pathological feature of the disease and not purely reactive. That does not exclude the contribution of environmental conditions to the incidence of affective symptoms as familial non-carriers also have higher than normal rate of symptoms compared to individuals without a family history of HD. However, longitudinal studies of psychiatric symptoms in later stages of manifest HD did not show a worsening or change in the percentage of HD patients that experience anxiety and depression. This further

supports that the incidence of these symptoms in genetic carriers are pathologically driven and less likely impacted by environmental conditions. Only apathy worsened with disease progression and is considered to be exacerbated by the worsening of the disease (Anderson and Marder 2001).

The most prominent affective disorder found in HD patients is major depressive disorder, exhibited by 20-64% of genetic carriers (Kirkwood et al. 2001; Paulsen et al. 2001; van Dujin et al. 2008; Reedeker et al. 2012), compared to the general population in which the lifetime prevalence is approximately 15%-20% (Narrow et al. 2002; Epping and Paulsen 2011), and 15-47% in non-carriers with a familial history of HD (Julien et al .2006; Martinez-Horta et al. 2016). Approximately 13%-71% of HD patients develop anxiety (Levy et al. 1998; Thompson et al. 2001; Paulsen et al. 2001; Dale et al. 2015). It is important to note that most studies identified a high prevalence of anxiety and depression in the same HD patient population, demonstrating that a significant portion of the patients experience comorbid anxiety and depression (Thompson et al. 2001, Paulsen et al. 2001; Duff et al. 2006; Kingma et al. 2008; Dale et al. 2016).

1.3.1 Etiology of affective disorders in HD

The neurobiology of the psychiatric symptoms of HD remains largely unknown given that the structural imaging studies that found a correlation between anatomical changes in the brain and cognitive and motor symptoms, did not find a significant correlation between cortical and striatal degeneration and the psychiatric symptoms in patients (Paulsen et al. 2008). This is despite the fact that the affective symptoms are present at the time that neurodegeneration is present in those regions. This suggests that

morphological alterations in the frontal-striatal loops in the brain are not sufficient to drive the manifestation of the psychiatric symptoms, but could be a factor of functional rather than anatomical changes in those or other circuits (Misiura et al. 2017).

A consideration to be made is that those structural studies do not inform the etiology of the psychiatric symptoms because the regions examined are not the sole mediators of mood and affect. Affect is primarily regulated by the limbic system, a network of cortical and subcortical structures that are functionally connected (Morgane et al. 2005). Furthermore, the limbic system is influenced by serotonin and norepinephrine, which are produced and released exclusively from brainstem nuclei. The status of brainstem nuclei was not examined in those studies. Therefore, additional studies are needed to directly examine structural changes in limbic regions outside the cortex.

A few studies did examine functional and structural changes in the serotonergic system in HD patients. In one study, researchers used transcranial sonography to identify potential alterations in raphe function in HD patients that had a history of depressive episodes or other psychiatric symptoms. They found evidence of reduced echogenicity in the dorsal raphe of the HD patients that displayed psychiatric symptoms and normal echogenicity in the raphe of HD patients that did not show psychiatric symptoms, which is suggestive of pathological changes in this structure due to the disease, and more importantly that this is correlated to the occurrence of affective disorders in HD (Krogias et al. 2011). Therefore, dysfunction of the serotonergic system is potentially implicated in the etiology of affective disorders in HD. It is most likely that serotonergic signaling is

impaired, given that pathological changes in serotonin function are associated with idiopathic affective disorders.

Serotonergic signaling was assessed indirectly in HD patients using numerous techniques. The first technique measured serotonin and the serotonin metabolite, 5-hydroxyindolacetic-acid (5HIAA) levels in the blood and cerebrospinal spinal fluid (CSF) of HD patients. Those studies observed no difference in either neurochemical levels in HD patients compared to controls or between HD patients with and without depression (Kurlan et al. 1988; Garrett and Soares-da-Silva 1992; Garcia Ruiz et al. 1995). However, this does not mean neurotransmitter function is not altered in HD as these measurements represent indirect indices of monoaminergic function. Furthermore, there are several factors that could affect the detection of neurotransmitters and metabolites in blood and CSF. Lastly, these findings are in contradiction to studies that did identify deficits in neurotransmitter systems in HD patients postmortem, which was a more direct measure of neurotransmitter levels in the brain.

Postmortem studies were conducted to investigate pathological changes in monoamine systems in HD patients with affective symptoms. Kish et al. (1987) quantified tissue levels of serotonin and serotonin metabolites in the caudate and putamen, nucleus accumbens and substantia nigra. Serotonin and 5HIAA levels were elevated in the caudate and putamen but not accumbens or substantia nigra. This increase in serotonin in striatum is supported by another study that also found increased tissue concentration of serotonin and 5HIAA in the striatum and pallidum in postmortem HD patients. Interestingly, this study also observed a slight decrease in serotonin levels and

slight increase in 5HIAA levels in hippocampus (Reynolds and Pearson 1987). Another study observed a similar phenomenon in which opposite effects on serotonin were observed in the same patient population. In this case, this study found evidence of increased serotonergic fibers in the striatum and decreased SERT activity in the nucleus accumbens of HD patients compared to controls (Bédard et al. 2011). An interesting dichotomy in these findings could be that serotonergic input is altered differently in the disease dependent on the target structure. Perhaps serotonergic inputs to limbic structures (hippocampus) are affected differently than serotonergic innervation of non-limbic structures (striatum). This remains to be investigated, which can be done using other empirical techniques to directly investigate the involvement of serotonin in the etiology of the psychiatric symptoms in HD.

1.4 Idiopathic affective disorders

Anxiety and depression can manifest behaviorally in several forms. The most common forms are as follows: 1) anxiety and depression exhibited as normal emotional states experienced by most individuals at one instance during their lifetime; 2) pathological anxiety and depression symptoms that make up another psychiatric or non-psychiatric condition; 3) anxiety and depression as a standalone mental illness (Klerman 1977). In the first instance, these behaviors are primarily reactive to environmental conditions or life hardships. In the second manifestation of anxiety and depression, the behaviors are considered secondary to a clinical condition. The third type is anxiety or depression present in absence of other medical conditions and presents as a single diagnostic entity. It can be beneficial to differentiate between the different presentations

of anxiety and depression as the neurobiology of each can differ. Particularly, the second and third presentations of these affective disorders are pathological in nature, whereas the first presentation is not a factor of neurobiological changes. In the case of HD, it is likely that the anxious and depressive symptoms best match the second presentation of affective disorders given that behavioral studies in HD patients identified an association between disease onset and psychiatric symptoms.

One of the earliest hypotheses on the neurobiology of affective syndromes, such as anxiety and depression, is the monoamine hypothesis (Klerman, 1977; Czeh et al. 2015; Hamon and Blier 2013). This hypothesis identified a deficit in activity in the neurotransmitter systems that comprise the monoaminergic dopamine, norepinephrine, and serotoninergic systems (Ressler and Nemeroff 2000). Of those neurotransmitter systems, serotonin and norepinephrine were strongly associated with the regulation of mood. This hypothesis emerged in the 1950s following the serendipitous discoveries that reserpine, used to treat hypertension, depletes neuronal stores of serotonin and norepinephrine and increases affective symptoms (Wehr and Goodwin 1977). The second discovery involved isoniazid, an antibacterial agent that reduced depressive and anxious symptoms in patients. Isoniazid blocks monoamine oxidase (MAO), the primary enzyme that metabolizes norepinephrine and serotonin. Blockade of MAO increases the synaptic availability of serotonin and norepinephrine. These significant events promoted the monoamine theory of depression and encouraged the development of therapeutics that increased monoaminergic activity in the brain This theory persists as one of the primary hypotheses of the neurobiology of psychiatric symptoms as the most common treatments for the disorders remain to be pharmacological agents that increase serotonin

or norephinephrine; with the most widely prescribed drugs being SSRIs (Ross et al. 2012). This is true for the treatment of psychiatric symptoms of HD as well (Martinez-Horta et al. 2016). Given the popularity of SSRIs in treating the affective disorders of HD, this implicates that anatomical and functional changes in monoaminergic systems, primarily the serotonergic system, underlies the pathophysiology of affective disorders in HD patients.

1.4.1 Serotonergic system in anxiety and depression

Serotonin is a neuromodulator and one of the primary neurotransmitters of the limbic system. Serotonin is produced and released exclusively from neurons originating from the raphe nuclei in the brainstem. The raphe is subdivided into nine subnuclei named B1-B9 (Tork 1990). The main subgroups are the dorsal and median raphe nuclei (Tork 1990). The projections from those two nuclei reach most major cell groups in the brain. The dorsal raphe projects to the prefrontal cortex, lateral septum, amygdala, dorsal striatum and ventral hippocampus (Hensler 2006). The median raphe projects to the dorsal and ventral hippocampus, medial septum, and hypothalamus. Those targets are the main brain regions, in addition to the raphe, which make up the serotonergic pathways that comprise part of the overall limbic system.

The dorsal (DRN) and median raphe nuclei (MRN) have a role in generating both anxious and depressive behaviors, which was discovered through activation and lesion studies (Andrade et al. 2013; Paul and Lowry 2013; Michelsen et al. 2007). This is in contradiction to other studies, which observed a distinct role of DRN in anxiety and MRN in depression (Hale et al. 2012; Spiacci et al. 2012; Almeida et al. 2013). Given

this discrepancy, it is feasible to speculate that the manifestation and regulation of affective disorders are not dependent on the robust activation or inactivation of the separate raphe nuclei but rather is dependent on activity in the postsynaptic targets of raphe (Nutt and Stein 2006).

Even though serotonin projections are widespread throughout the brain, not all of those projections are implicated in limbic function in healthy or HD patients. The serotonergic circuits crucial to regulating mood can be specified into 3 distinct circuits (Deakin and Graeff 1991). The first circuit originates in the DRN and projects to the dorsal and ventral hippocampus, subdivisions within the amygdala, and prefrontal cortex. This circuit is hypothesized to malfunction in anxiety disorders (Paul and Lowry 2013). The second pathway is implicated in panic disorders and will not be discussed, given that panic disorders are not a trait of HD. The third pathway originates in the MRN and projects to the septum and hippocampus. It is hypothesized that chronic stress increases serotonergic activity in this pathway, which dampens the behavioral response to stressful stimuli through the activation of postsynaptic 5-HT1A receptors (Paul and Lowry 2013). Failure of this pathway to inhibit responses to stressful stimuli is thought to result in depressive symptoms. This pathway is also implicated in anxiety disorders (Lowry et al. 2005). Unfortunately, these circuits have not been studied in depth in morphological, neurochemical or electrophysiological studies. As a result, there is mostly speculation as to how serotonin influences these circuits to manifest in affective disorders.

Given the vast networks that make up the limbic system, my dissertation focused on one key circuit that is implicated in both anxiety and depression, which was the raphe-

ventral hippocampal circuit. Therefore, the remainder of my dissertation will be limited to the discussion of that circuit.

1.4.2 Hypothesized role of raphe-vHPC projections in affective behavior

The hippocampus can be isolated into two functionally separate structures; the dorsal and ventral hippocampus (Fanselow and Dong 2010).The two subdivisions of hippocampus have different behavioral functions. Lesions of the dorsal hippocampus (dHPC) impair memory retention and spatial performance in tasks such as the Morris water maze whereas lesions of the vHPC do not affect spatial memory performance (Kjelstrup et al. 2002; Bannerman et al. 2003; McHugh et al. 2004). Lesions of the vHPC elicit an anxiolytic effect in tests of unconditioned anxiety such as the elevated plus maze (Kjelstrup et al. 2002), the light-dark box test (Bannerman et al. 2003, McHugh et al. 2004), social interaction task (McHugh et al. 2004), in exploration of novel environments (McHugh et al. 2004) and reduce freezing behavior in tests of conditioned anxiety (Bannerman et al. 2003). The chemogenetic inactivation of the ventral hippocampus but not dorsal hippocampus prevented the consolidation of contextual fear memories (Zhu et al. 2014). As a result, the existing functional dichotomy of hippocampus is that the dorsal hippocampus is involved in non-aversive memory formation and the ventral hippocampus is primarily involved in limbic function including the storage and processing of fear-associated memory. The vHPC is a widely connected structure with afferents from several limbic structures such as the prefrontal cortex, amygdala, the DRN and MRN (Hensler 2006) with reciprocal efferents to those same structures. This connectivity of vHPC supports its role in regulating limbic function.

For the scope of this dissertation, I was interested in serotonergic control of the vHPC as it is implicated in anxiety and depression. Microdialysis is a useful technique to examine direct neurochemical changes in the brain as related to behavior (Kirby and Lucki 1995). Following exposure to anxiety-inducing stimuli, it has been shown that serotonin efflux is increased in the vHPC (Amat et al. 1998, Kagamiishi et al, 2003). This suggests that increased serotonergic efflux to vHPC is anxiogenic. Interestingly, extracellular serotonin in the vHPC slightly decreases following the forced swim test, which suggests that serotonin activity is reduced in response to stressful stimuli (Kirby and Lucki 1991). Based on the monoamine hypothesis of anxiety and depression, this response is expected as it is hypothesized that increases in serotonin are anxiogenic and a decrease in serotonin has a depressant effect (Gordon and Hen 2004). However, these experiments measured the evoked changes in serotonin activity to anxious and depressive stimuli in rodents that do not exhibit anxious or depressive symptoms *de novo*. Therefore, this offers little indication as to the baseline physiological condition of the serotoninergic system in an animal model of anxiety or depression. No studies to date have examined serotonin efflux in an animal model of comorbid anxiety or depression, only in animal models that exhibit only anxious behaviors or only depressive behaviors. Therefore, it remains unknown how serotonin efflux can manifest in this comorbid behavioral phenotype. I sought to investigate this in HD using a translational mouse model of the disease.

1.5 Translational models of Huntington's disease

After the genetic cause of the disease was discovered in the 1990s, animal models were generated to express the genetic mutation in the HTT gene. The first transgenic mouse model was reported in 1996 (Mangiarini et al. 1996). Subsequently, other transgenic mouse models and another type of genetic model, the "knock-in" mouse model, were created in the last 30 years (Menalled and Chesselet 2002). The transgenic mouse models express a transgene containing either a fragment (exon 1) or the full-length human HTT gene. The fragment transgenic mouse model typically expresses a higher CAG repeat length (~150), which results in a rapid progression of HD in the rodents and early death (~3 months old). The transgenic mouse models, express no more than 130 repeats, all introns and exons of the gene, under the control of the human HTT promoter. This results in a slower progression of the disease and a longer life-span. As a result of the method used to insert the HTT gene in the mouse genome, all transgenic mouse models express three copies of the gene, the two endogenous mouse HTT gene and the mutant human copy of the gene. In addition to transgenic mouse models, which were created using yeast or bacterial artificial chromosomes, knock-in mouse models were generated. The knock-in mouse models only have two copies of the endogenous mouse gene with the addition of an increased CAG repeat in the precise location of the polyQ chain the mouse HTT gene. The approximate CAG repeat length of the knock-in models ranges from 111-130 repeats similar to the full-length transgenic mouse models. Both of these models exhibit a slow disease progression and near normal life span for the rodents. In this study, all experiments were conducted in a transgenic mouse model.

1.5.1 BACHD transgenic mouse model of HD

Since the creation of these models almost thirty years ago, hundreds of studies have demonstrated the usefulness of investigating rodent HD models in preclinical studies. This usefulness was determined by revealing the numerous similarities between human HD and the HD mouse models, primarily regarding the behavioral progression of the disease and the underlying pathology (Gray et al. 2008; Slow et al. 2003).

The BACHD mouse model is a transgenic mouse model that was generated on the FVB/NJ mouse background. The human HTT gene was inserted in fertilized eggs of FVB mice using a 240 kb bacterial artificial chromosome (BAC) that contains the 170 kb human HTT locus (Gray et al. 2008). The human HTT gene was modified to express 97 CAA/CAG repeats in exon 1 of the gene and then inserted into the mouse genome using the BAC. Similar to HD patients, this mouse model has mutant HTT protein aggregates present in the cortex, striatum, and cerebellum. Other pathological similarities include atrophy of forebrain cortical areas and decreased striatal volume that is attributed to the specific degeneration of striatal medium spiny neurons (Gray et al. 2008). Additionally, this mouse model recapitulates several of the behavioral changes that are observed in the human condition, including progressive motor deficits.

1.5.2 Affective behaviors in HD transgenic mouse models

Once affective disorders were identified in HD, researchers began investigating the cognitive and psychiatric abnormalities in animal models in order to find the underlying cause. The most widely studied transgenic mouse models of Huntington's

disease include the R6/2 model, R6/1 model, YAC128 model, and the BACHD model. Several studies have revealed that these models show anxiety-like and depressive-like symptoms (Abada et al. 2013; Hickey et al. 2008; Menalled et al. 2009; Pang et al. 2009; Pouladi et al. 2009; Pouladi et al. 2012; Renoir et al. 2011). Interestingly, the BACHD mice exhibit a phenotype of comorbid anxiety and depression (Gray et al. 2008, Menalled et al. 2009). Thus, the BACHD mouse model is a potential useful model for studying the pathophysiology underlying these mood abnormalities in HD.

1.5.3 Etiology of affective disorders in HD transgenic mouse models

These mouse models are beneficial for studying the incidence of affective disorders in HD because many of the models exhibit behavioral abnormalities that resemble anxiety and depression. Some studies have examined these symptoms further by investigating how monoamine function is affected in these HD mouse models. Monoamine function was assessed in the mouse models because SSRIs remain the first-choice treatment for the mood abnormalities experienced by patients. Additionally, monoamine dysfunction was also evident in the symptomatic HD patients. The studies conducted to date found decreased tissue concentration of serotonin, dopamine, noradrenaline and their metabolites in the striatum, cortex and hippocampus (Garcia-Miralles et al. 2016; Renoir et al. 2011), diminished functional binding of serotonin receptors including the 5-HT1A and 5-HT2A receptor (Renoir et al. 2011; Yohrling et al. 2002), as well as reduced serotonin transporter and tryptophan hydroxylase expression in the raphe (Renoir et al. 2011). Those findings further support that the serotonergic system undergoes pathological changes in HD mouse models, similar to the human condition. It

is important to note that the tissue concentrations of the monoamines and metabolites were analyzed postmortem, which is an indirect method of measuring functional activity of monoaminergic networks relative to existing *in vivo* methods such as microdialysis. Therefore, the functional status of monoamine systems should be examined *in vivo* in HD transgenic mouse models to support or provide further evidence of altered monoamine activity.

1.6 Thesis objective

One goal of my dissertation was to identify an animal model of Huntington's Disease that is a valid translational model for the study of the psychiatric symptoms of HD. This will be assessed using the three principles of validity: face, predictive and construct validity. Face validity will be determined if the BACHD mouse model encompasses the behavioral phenotype of the psychiatric symptoms of HD. The criterion for predictive validity is dependent on the mouse model exhibiting a similar response to pharmacological treatments used to alleviate symptoms in HD patients. The construct validity of the mouse model is dependent on determining if the underlying etiology of the symptoms in the mouse model matches what is known about the etiology of the symptoms in patients. Additionally, the identification of a valid translational mouse model will enable new studies into the pathophysiology of the psychiatric symptoms of HD.

Chapter II:

General Methods

2.1 Subjects

Transgenic male BACHD mice (FVB/N-Tg(HTT*97Q)IXwy/J) and their age-matched wildtype controls were obtained from The Jackson Laboratories (Bar Harbor, ME, USA) at 5-6 weeks old. The BACHD transgenic mouse model is a Bacterial Artificial Chromosome (BAC) - mediated HD model that expresses the full-length human mutant huntingtin gene with 97 CAA/CAG repeats under the control of the endogenous mouse htt regulatory machinery (promotor, all introns and exons) (Gray et al. 2008). Animals were housed individually in plastic shoebox cages with food and water available ad libitum at all times. Animals were kept in a colony that was maintained at 21 °C with a 12 h light/dark cycle (lights on at 0700 h). All efforts were made to minimize animal suffering and to limit the number of animals utilized for these experiments. Animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Rutgers University IACUC. Prior to all behavioral tasks, the mice (BACHD mice and wildtype controls) were handled for 5 minutes a day for 5 consecutive days. This was completed in effort to reduce any stress by handling in subsequent behavioral tests.

2.2 Measure of motor performance

The fixed-speed rotarod task and spontaneous activity in the home cage and in an activity chamber were used as measures of motor coordination ability and activity levels respectively.

2.2.1 Longitudinal measure of motor performance

The fixed-speed rotarod task was used to monitor motor ability longitudinally in the BACHD mice and control wildtypes. The mice were tested at 8, 12, 16, 20, and 24 weeks old. The rotarod task required training prior to testing. The training and testing protocols are detailed in the following data chapter.

2.2.2 Acute test of motor performance

In a separate experiment, BACHD mice and wildtype mice were tested a single time on the fixed rotarod task to ensure they did not show motor impairments that could impair performance in subsequent tests for the anxiety and depressive phenotype. Additionally, spontaneous behavior was used to identify any deficits in activity levels in the mice. This was completed in the home cage and in an activity chamber. Activity was measured in both test conditions using a 4 x 9 infrared photobeam system (PAS systems, San Diego, California, USA). Activity was measured as number of times the animal crossed one photobeam.

2.3 Behavioral tests of affective phenotype

The tests for anxiety-like and depressive-like behaviors were completed in the duration of a week when the mice were 8 weeks old. Each mouse underwent a series of behavioral tasks to investigate the extent of behavioral abnormalities exhibited by the BACHD mice and controls. This included the forced swim test (FST), the Tail Suspension Test (TST), the Open Field Test (OF) test and the elevated zero maze (EZM). All behavioral tasks were completed during the light cycle.

2.3.1 Measure of depressive-like behavior

The depressive-like behavior of both the WT and BACHD mice was assessed using the Forced Swim Test. The FST apparatus was a clear Plexiglas cylinder (Diameter 10 cm, Cylinder height 30 cm, Water height 20 cm). Each trial for the FST was six minutes. The first minute is to allow the animal to adjust to swimming. The final five minutes are scored for length of time spent immobile (in seconds). The tail suspension was conducted by suspending the mouse by the tail using tape. The trial for both the TST and FST are videotaped and scored at a later viewing. Additional details of the test parameters are included in the relevant data chapter.

2.3.2 Measure of anxiety-like behavior

The anxiety-like behavior of both the WT and BACHD mice was assessed using the Elevated Zero Maze task. The Elevated Zero Maze (Stoelting, Wood Dale, IL, USA) is a circular maze (50 cm in diameter) with two enclosed arms and two open arms (lane width 5 cm, wall height 15 cm). The maze is elevated 40 cm. The illumination of the maze is controlled, with the open arms illuminated at 100-120 lux, and the closed arms illuminated at 25-50 lux. Trials in the elevated zero maze lasted five minutes. The trial is initiated by placing the mouse into a closed arm. The entire trial is videotaped. Trials are then watched and scored for the latency to enter the open arm, the number of head dips, the number of open arm entries, and the length of time spent in an open arm. Additionally, anxiety-liked behavior was measured in the Open Field Test. The maze used in the open field consisted of a 40 x 40 cm gray plastic box. The center was designated as a 20 x 20 cm square in the arena. The center was brightly illuminated to

~200 lux. The trial lasted five minutes and was started when the mice were placed in the corner of the open field. The distance traveled in the entire maze, the center of the maze, and the time spent in the center of the maze was tracked and calculated using Noldus Ethovision (Noldus, Netherlands).

2.4 Effect of acute SSRI and 5-HT1A agonist treatments on anxiety-like and depressive-like behavior

Using the FST and EZM, the effect of acute, systemic administration of the selective serotonin reuptake inhibitor (SSRI) escitalopram and the 5-HT1A receptor agonist, 8-OH-DPAT, on the depressive and anxious phenotype respectively of the mice was tested. The testing parameters were the same for testing with and without drugs. Mice were tested in the FST and EZM 30 minutes and 60 minutes following subcutaenous injection of 8-OH-DPAT (0.3 mg/kg, 3.0 mg/kg) and intraperitoneal injection of escitalopram (1 mg/kg) respectively. Control animals received vehicle injections and were tested 30 minutes or 60 minutes after injection. Control mice were tested following the same wait time as animals injected with 8-OH-DPAT or escitalopram (relevant to which drug was being tested at the time). In addition, mice were tested in these drug experiments twice – once in FST and once in EZM. All testing was done in the EZM prior to testing in the FST, the more stressful of the two tests. The mice either received vehicle in one test and either escitalopram or 8-OH-DPAT in the other behavioral test. All trials were counterbalanced so that mice either received vehicle or a drug treatment for the first test.

2.5 *In vivo* microdialysis procedure

Following the completion of all behavioral experiments, the mice underwent microdialysis experiments to measure neurotransmitter efflux. Neurotransmitter efflux is the local concentration of neurotransmitter available in the extracellular space that is in proximity to several synapses. Neurotransmitter efflux, at any given time, is determined by neurotransmitter release, reuptake, receptor, binding, metabolism, and diffusion taking place in multiple synapses. All mice were tested in microdialysis experiments between the ages of 9-12 weeks. This reflects the start of dialysis experiments approximately a week after the completion of behavioral experiments and the end of experiments, which included the approximately two-three weeks it took to complete surgery and experiments in 6-12 mice per cohort (~4 mice/week).

Microdialysis probes consisted of a vertical, concentric design similar to that previously described in our laboratory (Cobb and Abercrombie, 2002). Probes were constructed locally, such that the probe inlet consisted of piece of PE-10 tubing (Clay Adams, Parsippany, NJ, USA), whereas a piece of fused silica capillary tubing (I.D. 75 μm and O.D. 150 μm; Polymicro Technologies, Phoenix, AZ, USA) served as the outlet. A semi-permeable microdialysis membrane (molecular weight cut-off =13,000; O.D. = 220 μm; Spectrum Laboratories, Rancho Dominguez, CA, USA) was placed over the end of the exposed silica tubing, glued to the PE-10 tubing and coated with a thin epoxy layer, leaving a 2 mm long active exchange area at the end of the probe. Prior to implantation, probes were calibrated in vitro to determine their relative recovery rates. Only probes with recovery rates between 10 and 15% were used.

2.5.1 Stereotaxic surgery

Mice were anesthetized with 80-100 mg/kg ketamine and 8 mg/kg xylazine (i.p.) and mounted into a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The skull was exposed and adjusted until flat, a craniotomy was performed at the specific coordinates for the target region, unilateral probes were slowly lowered (by 1 mm/min) into the desired region at the following coordinates: striatum: ML – ±2.5; AP – -3; DV – -4; ventral hippocampus: ML – ±3; AP – -2.7; DV – -4. Probes were secured to the skull with super glue, dental cement and a plastic head cap. Once the cement is dried, probes were continuously perfused with artificial cerebrospinal fluid (aCSF; NaCl 147 mM, KCl 2.5 mM, CaCl2 1.3 mM, MgCl2 0.9 mM, pH 7.4) using a microliter infusion pump (Harvard Apparatus, Holliston, MA, USA) driving gastight glass syringes (5.0 ml; Hamilton, Reno, NV, USA) at a flow rate of 1.5 µl/min. Probe tubing was threaded through a fine wire tether, which was attached to a multi-axis counter-balanced lever arm and the probe inlet was connected to a fluid swivel (Instech Laboratories, Plymouth Meeting, PA, USA) to allow the mice to move freely. Dialysate samples are collected during the light portion of the light-dark cycle, 16-18 hours after the completion of surgery.

2.5.2 Measure of serotonin and dopamine efflux in striatum and ventral hippocampus

Dialysate samples (20 microliters) are collected in 15-minute intervals and immediately frozen with dry ice and stored at -80°C until assayed for dopamine or

serotonin using high performance liquid chromatography (HPLC) with electrochemical detection. Samples are collected the following day after surgery (16-18 hours later). All efforts were made to collect samples during the light cycle. Additionally, all baseline samples were collected around the same time during the light cycle: 11 am – 3 pm. All drug experiments using microdialysis were conducted between 3 pm – 7 pm. Serotonin was quantified using the HTEC-500 system equipped with a PP-ODS II or CAX column (Eicom USA, San Diego, CA, USA). This standalone system was equipped with the column oven, pump and electrochemical cell that applied a potential of +450 mV. The cell consisted of a graphite electrode (WE-3G) and reference electrode with an AgCl conduction solution (Eicom USA, San Diego, CA, USA). Mobile phase was delivered through the system at a flow rate of 0.5 ml/min (PP-ODS II) or 0.25 ml/min (CAX). Mobile phase for the PP-ODS II column consisted of 0.1 M Phosphate buffer (pH 5.4), 1.5% methanol, 50 mg/L decansulfonate sodium salt, and 50 mg/L Ethylendiamine-N, N, N', N-tetraacetic acid disodium (EDTA-2Na). Mobile phase for the CAX column consisted of 0.1 M Ammonium acetate buffer (pH 6.0), 30% methanol, 0.06 mol/L sodium sulfate, and 50 mg/L EDTA-2Na. DOPAC and 5HIAA (monoamine metabolites) were quantified using a separate HPLC system that consisted of an injector (Rheodyne, Cotati, CA, USA), a VeloSep RP-18 column (100 × 3.2 mm; PerkinElmer, Waltham, MA, USA) and a Shimadzu LC-10AD VP solvent delivery pump (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA), that delivered the mobile phase at a flow rate of 0.8 ml/min. The mobile phase was composed of 0.1 M sodium acetate buffer (pH 4.5), 0.1 mM EDTA-2Na, 1.2 mM sodium octyl sulfate, and 8% methanol. An electrochemical detector (Coulochem II; ESA Inc., Chelmsford, MA, USA) with a flow cell electrode set

at an applied potential of +400 mV was used. The detector output for both HPLC systems was connected to a computerized data acquisition system (PowerChrom, Denistone East, NSW, Australia). Baseline levels of each neurotransmitter and metabolite will be determined from 3 dialysate samples within ± 15% variability.

2.5.3 Measure of neurochemical response to local infusion of SSRI in vHPC

Serotonin efflux was measured for one hour (4 samples) during the local administration (via reverse dialysis) of escitalopram (10 μM, 1μM, and 0.1 μM dissolved in aCSF) and for one hours following the removal of escitalopram from the aCSF bath and administration of aCSF without drug.

2.5.4 Histology for Probe Placement:

Following the conclusion of the dialysis experiments, mice are sacrificed with an overdose of chloral hydrate and perfused transcardially with 0.9% saline, followed by a 4% paraformaldehyde and 15% picric acid solution. Brains were post-fixed in fresh fixative for 24 hours followed by long-term storage in 30% sucrose until sectioned. 60 micron sections were taken of the striatum and ventral hippocampus and stained with Neutral Red to confirm probe placement in the targeted regions.

2.6 Timeline of experiments

The behavioral tasks are completed in the span of one week when the mice are 8 weeks old. All microdialysis experiments are started 3 days after the completion of behavioral studies, when the mice are 9 weeks old. Usually, up to four microdialysis

experiments are conducted a week. As such, the approximate age of the mice during dialysis experiments is 9-12 weeks due to the length of time required to conduct experiments in one cohort of 6-12 mice. Surgeries are conducted in a counterbalanced manner to ensure an equal number of wildtypes and BACHD mice are tested at each age point during the 9-12 week old time period.

2.7 Statistical analysis

Behavioral and dialysate data is presented as mean ± SEM. Dialysate values represent picograms per 20 µl dialysate sample. 2-way factorial ANOVA was used to assess main effect in the rotarod task, and the effect of drug on behavior and serotonin efflux. Data were subdivided according to the interactions found in the global test and separate lower order analyses were carried out. Where appropriate, posthoc testing using the Bonferroni correction for multiple comparisons was used. The student's t test was used to analyze the performance between WTs and BACHD mice in the Forced Swim Test and Elevated Zero Maze. The student's t test was used to analyze differences in serotonin, dopamine and metabolite levels, as well as area under the curve (AUC) values between WTs and BACHD mice. Differences were considered statistically significant when $p < 0.05$.

CHAPTER III:

BACHD mice exhibit anxiety-like and depressive-like symptoms in select tests of affective behaviors prior to robust motor symptoms

3.1 Rationale:

Affective disorders in Huntington's Disease (HD) remains an important area of focus in translational and clinical research, as little has been discovered about the underlying pathophysiology of these symptoms (Pla et al. 2014). The onset of psychiatric symptoms occurs in the prodromal or presymptomatic stage of HD (Anderson and Marder 2001). During this stage, HD patients exhibit little to no motor deficits, but have been shown to exhibit psychiatric-like symptoms such as anxiety and depression (Epping et al. 2016). It is difficult to study the neural basis of these symptoms in patients without the use of invasive techniques. Fortunately, translational mouse models of Huntington's Disease can be utilized to study the neurobiology of the disorder. A challenge of using a translational animal model is ensuring that they mimic certain aspects of the human disease. The standard way to assess the validity of using a particular animal model of a human disease is to determine the face, predictive and construct validity of the model (Feusner et al. 2009).

Face validity is the concept that the animal model displays symptoms analogous to the human condition. In the case of the early affective stage of HD, an animal model meets the criteria for face validity if they exhibit the two most common psychiatric symptoms of HD, anxiety and depression. The second criterion for the validation of an animal model is predictive validity, which states that the analogous symptoms of interest should be ameliorated by the same treatments that are shown to be effective in the human condition. The final criterion for the validation of an animal model is construct validity,

which is the concept that the animal model has a similar pathophysiology of the disease or symptoms as compared to the human condition.

The primary objective of this chapter is to investigate the face validity of the early behavioral motor and non-motor characteristics of translational HD mouse models. No studies have examined if HD mouse models behaviorally reflect the prodromal stage of HD in which the non-motor symptoms manifest prior to the motor symptoms. To address this, I examined if a transgenic mouse model of HD meets the following criteria of the prodromal stage of HD: 1) display little to no motor deficits and 2) display anxiety-like and depressive-like behaviors concurrently. I conducted these studies in the BACHD transgenic mouse model. This mouse model exhibits motor deficits, neuropathological traits of the disease, as well as psychiatric-like symptoms, similar to traits observed in patients (Gray et al. 2008).

In order to determine the age at which the early affective studies should be conducted in the BACHD mice, I needed to identify an age in which the mice exhibit little to no motor deficits. Menalled and colleagues (2009) identified that the BACHD mice begin to show motor deficits on the rotarod task at 4 weeks old. This suggests that the onset of motor symptoms occurs at this age. However, in that same study, rotarod performance in the same cohort of mice improved at later ages (8 and 12 weeks old) and did not show a decline in performance again until 28 weeks old. Therefore, it remains unknown if the progressive motor deficits emerge at 4 weeks old or later at 28 weeks. Another study reported the onset of motor impairment in the rotarod task at 8 weeks old with a progressive decline in performance at 24 weeks old (Gray et al. 2008). A third

study identified the onset of progressive deficits in rotarod performance at 16 weeks old (Pouladi et al. 2012). Pouladi and colleagues also tested rotarod performance at 8 weeks but did not identify a deficit in motor ability. Therefore, the time course of the onset of motor impairment is debatable but is considered to emerge between 8 weeks to 16 weeks of age. This remains to be investigated. Using a similar protocol, I longitudinally monitored motor performance on the rotarod task in the BACHD mice to determine the onset of motor impairment at ages tested previously in other studies.

Therefore, prior to testing the psychiatric symptoms in the mice, I sought to progressively test the motor performance of the BACHD mice in monthly intervals, at 8, 12, 16, and 24 weeks old. I identified that deficits in rotarod performance are moderately present at 12 weeks old and robustly manifests at 16 weeks old (shown in Figure 3.1). Consequently, I selected to examine all non-motor behavioral changes prior to 12 weeks old.

Few studies have examined the incidence of affective disorders in the BACHD mouse model. Menalled and colleagues (2009) reported that the BACHD mice show a preference for the dark compartment during the light-dark box test at 4, 8, 12, 16 and 26 weeks old, which is suggestive of that anxious behavioral phenotype of BACHD mice is first present at 4 weeks old. Additional studies examined the prevalence of affective behavior in the BACHD mice but at a much older age. In one study, it has been shown that the BACHD mice show a depressive phenotype at 12 months old (Pouladi et al. 2012). Similarly, another study observed that older (9-10 month old) BACHD mice exhibit an anxious phenotype (Abada et al. 2013). However, those studies only tested for

evidence of only one behavioral phenotype - anxiety or depression. Importantly, anxiety and depression are often expressed comorbidly in HD patients (Martinez-Horta et al. 2016). Furthermore, these studies were conducted at an age in which the BACHD mice show motor deficits, which can impact their performance in anxiety and depressive tests. Thus, the incidence of affective disorders in the BACHD mice should be determined in the mice prior to the known onset of the motor deficits. Additionally, given that human HD patients experience anxiety and depression in conjunction with few motor deficits, I characterized the progression of both the motor and psychiatric-like symptoms in the BACHD mice prior to 12 weeks old, when motor symptoms are mildly present.

To summarize, the experiments in this chapter aimed to determine if there is an observed psychiatric-like phenotype in the BACHD mice at a young age (before 12 weeks old). These experiments consisted of behavioral studies of motor symptoms, anxiety-like and depressive-like symptoms.

3.2 Brief Overview of Methods:

In this study, I assessed the motor and affective abnormalities in the BACHD mouse model. All data collection began when the mice were 7 weeks old and was completed by 8 weeks of age (for all behavioral tasks). The behavioral tasks took approximately two weeks to complete as all mice underwent motor tests in addition to the affective tests. The order in which the motor tests and affective tests were administered was counterbalanced between cohorts of mice. Therefore, some cohorts were tested on motor behaviors first and affective tests in the following week, with the remaining cohorts tested in the opposite order. This ensured that the approximate age for testing was

similar for the motor and affective tests. However, I did control the order of the affective tests in order to prevent the stress of the tail suspension test and forced swim test from affecting the anxiety measures. The tests were administered in the following order: 1) Elevated Zero Maze, 2) Open Field Test, 3) Tail Suspension Test, 4) Forced Swim Test. A minimum of 24 hours elapsed between all tasks with only 1 task per day.

To briefly summarize, the behavioral tasks used included the fixed-speed rotarod task of motor coordination, spontaneous locomotor activity, open field (OF) test, elevated zero maze (EZM), the modified forced swim test (FST) and the tail suspension test (TST). The rotarod task required two days of training and one day of testing. Training consisted of 5 trial runs at 10 rpm for 60 seconds on two consecutive days. The testing consisted of two trial bouts with a 60 second run at each of the 5 different speeds: 10, 15, 20, 25, and 30 rpm. There was a 5-minute intertrial interval and 60 seconds between each run. In the longitudinal study, BACHD mice and wildtype controls were tested on rotarod performance at 8, 12, 16, and 24 weeks old, with a similar training and testing paradigm, except mice were tested on 10, 20 and 30 rpm only. Locomotor testing in the activity chamber required two days of habituation for 1 hour and testing for 1 hour on the third day. Locomotor testing in the home cage was measured for 1 hour on a separate day. The OF test lasted 1 hour and was scored for time spent in the open center of the test arena, and percentage of the total distance traveled in the center. The EZM was a 5 minute trial in which behavior was scored for latency to enter the open arm, the total time spent in the open arm, number of head dips, and the number of entries in the open arm. The trial time for the FST and TST was 6 minutes. The final 5 minutes were scored for the total time spent mobile. All analyses of the TST and FST were conducted on the time spent

immobile, which was the difference between the total trial time and the scored mobility time.

3.3 Results:

A total of 20 WT male mice and 20 BACHD male mice were tested for motor ability and affective tests at 8 weeks old. The mice were tested in 4 cohorts (Cohort 1: n = 6 WT and 6 BACHD mice; Cohort 2: n = 6 WT and 6 BACHD mice; Cohort 3: n = 4 WT and 4 BACHD mice; Cohort 4: n = 4 WT and 4 BACHD mice). Only 3 of those cohorts were tested in the locomotor chambers as an additional measure of motor performance, thus accounting for the discrepancy in the count for the rotarod and locomotor experiments. The primary objective of these experiments was to characterize the symptomatic and pathological progression of early-stage HD in a mouse model. Table 3.1 summarizes the approximate age at which the mice underwent the motor tasks. Table 3.2 shows the approximate age of mice when they were tested in the battery of affective tests. This data was collected from 4 cohorts of mice. The open field test and tail suspension test were added as an additional measure of affective behavior in 3 cohorts of mice in later experiments. The motor tests and affective tests were conducted around the same age in both BACHD and WTs.

	Rotarod Task		Locomotor Behavior – Activity Chamber		Locomotor Behavior – Home Cage	
	WT	BACHD	WT	BACHD	WT	BACHD
Age	56.00 ± 0.97	55.00 ± 0.65	53.00 ± 0.30	54.17 ± 0.68	49.50 ± 0.45	50.67 ± 0.83
N	20	19	16	16	16	16

Table 3.1 Approximate age and number of WT and BACHD mice tested for motor abnormalities. All tests for motor ability began at approximately 50-56 days old (~8 weeks old).

	Elevated Zero Maze		Open Field Test		Forced Swim Test		Tail Suspension Test	
	WT	BACHD	WT	BACHD	WT	BACHD	WT	BACHD
Age	54.6 ± 1.23	53.63 ± 1.07	53.50 ± 1.55	53.67 ± 1.05	55.2 ± 1.24	54.11 ± 0.84	55.71 ± 1.93	54.46 ± 1.54
N	20	19	12	12	20	19	14	13

Table 3.2: Approximate age and number of WT and BACHD mice tested for affective symptoms. All mice underwent behavioral testing for affective symptoms at approximately 53-55 days old (~8 weeks old) in the time span of one week.

3.3.1 Progressive deterioration of BACHD mice motor performance on fixed speed rotarod task

Figure 3.1 depicts the rotarod performance of BACHD mice and WT mice, tested at 8, 12, 16 and 24 weeks old. The mice were tested on three speeds: 10, 20 and 30 rpm. Rotarod performance at each speed was analyzed separately using a 2-way ANOVA comparing age and genotype followed by multi-comparisons posthoc testing using the Bonferroni alpha-correction method. For 10 rpm, a 2-way ANOVA revealed genotype differences [$F_{1, 48} = 16.49$, $p = 0.0016$] in rotarod performance and a significant Genotype x Age interaction [$F_{4, 48} = 3.021$, $p = 0.0266$]. At 10 rpm, multiple comparisons posthoc tests using the Bonferroni correction determined that BACHD mice performed worse than WTs at 20 weeks old. For 20 rpm, a 2-way ANOVA revealed genotype differences [$F_{1, 48} = 9.802$, $p = 0.0087$]. Multiple comparisons posthoc tests using the Bonferroni correction determined that BACHD mice performed worse than WTs at 16 and 20 weeks old. For 30 rpm, 2-way ANOVA revealed genotype differences [$F_{1, 48} = 22.58$, $p = 0.0005$]. Multiple comparisons posthoc test using the Bonferroni correction determined that BACHD mice performed worse than WTs at 16, 20 and 24 weeks old. From this study, I selected 8 weeks old as the starting age for all behavioral experiments.

3.3.2 BACHD mice show motor impairments at 8 weeks old

In subsequent experiments, mice were tested on rotarod performance at 8 weeks old. This was done to confirm the findings from the longitudinal study (detailed in section 3.3.1) that the BACHD mice do not show severe motor deficits at 8 weeks old. It was important to ensure that any presence of motor impairment would not occlude the

mice's ability to complete the affective tests. This was completed in a separate cohort of mice that was tested on rotarod, in the EZM, OF test, TST and FST. Using the fixed-speed rotarod, I tested the motor coordination ability of the BACHD mice and age-matched wildtype controls by measuring the latency to fall off a rotating rod in a 60-second period, at various speeds with increasing difficulty. A 2-way ANOVA of rotarod performance (Figure 3.2) revealed a significant Speed x Genotype interaction ($F_{4.37}$ = 7.450, $p < 0.0001$). Additionally, there was a significant effect of speed ($F_{4,37}$ = 3.783, $p < 0.0001$) and genotype ($F_{1,37}$ = 13.25, $p = 0.008$). However, in order to accurately determine if rotarod performance of the BACHD declines, I decided *a priori* to only compare the performance of the BACHD mice to the WTs at each speed. In order to do this, I ran an individual 2 sample t-test comparing the average latency to fall between groups at each speed. I adjusted the experiment-wise alpha since these are separate t-tests on measures conducted in the same test. The alpha value was adjusted to $p = 0.01$ (0.05/5 tests). Our results show that the BACHD mice fell off at a shorter latency, relative to WTs, at 25 rpm and 30 rpm (Fig. 3.2, $p = 0.0077$ and $p = 0.0017$ respectively). This is indicative of impaired motor coordination at higher (and more difficult) speeds. In addition to utilizing the fixed-speed rotarod task for tests of motor ability, I measured spontaneous locomotor activity in the home cage and in an activity box for 12 BACHD mice and 12 age-matched WT controls. There was no discernable difference in the level of spontaneous locomotor activity between the WTs and BACD mice in either the activity box or the home cage (Figure 3.3: Home cage: t_{30} = 0.410, $p = 0.685$; Activity box: t_{30} = 1.283, $p = 0.209$). Spontaneous locomotor activity was also assessed in the open field test by measuring the total distance travelled in the open field. The total

distance travelled in the open field did not differ between groups [Fig.3.6, $t(22) = 0.962$, $p = 0.346$).

Given the impairment in motor coordination on the rotarod task, and the lack of a difference in spontaneous activity between genotypes, suggests that the BACHD mice exhibit minimal motor deficits at this age.

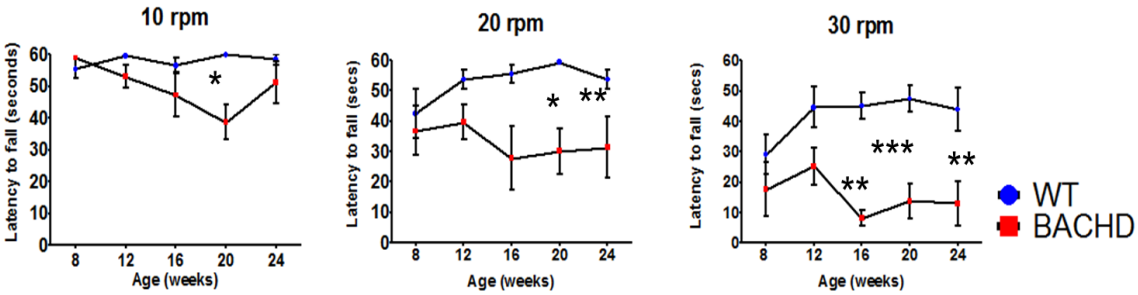

Figure 3.1: Progressive motor deficits in BACHD mice on fixed-speed rotarod task. At more difficult speeds, BACHD mice begin to show impairments in motor coordination starting at 16 weeks. This was crucial to determine as I was targeting an age in which the BACHD mice do not show robust motor deficits. For this reason, I completed all testing from 8 – 12 weeks old. *, **, ***: between genotype differences; $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively.

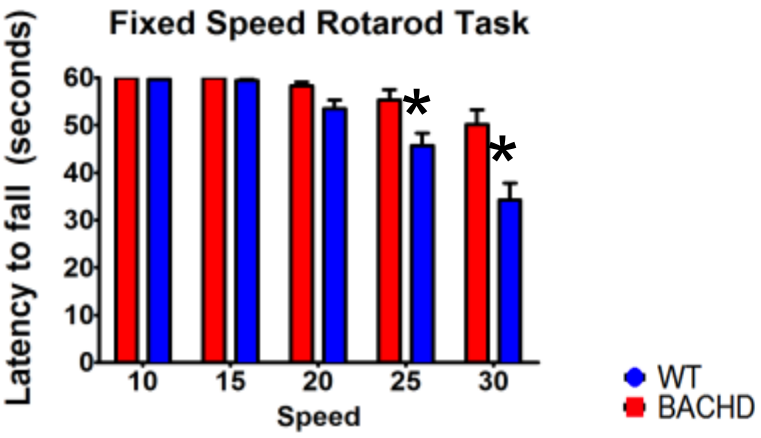

Figure 3.2: Motor coordination in Fixed-Speed Rotarod Task at 8 weeks old. At 8 weeks old, the BACHD mice show a subtle impairment in motor ability on the rotarod task at higher speeds (25 and 30 rpm) relative to age-matched healthy controls (WT). *: $p < 0.01$

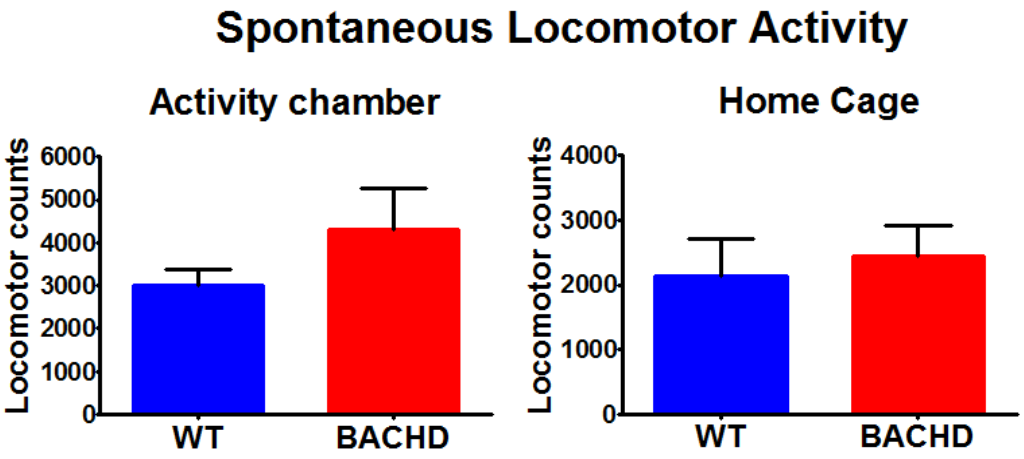

Figure 3.3: Spontaneous Locomotor Behavior in Home Cage and Activity Monitoring chamber. There were no significant differences between spontaneous locomotor activity of the BACHD mice compared to WTs in either the activity box or the home cage.

3.3.3 BACHD mice exhibit anxiety-like and depressive-like symptoms

I utilized a battery of four behavioral tasks to assess the prevalence of anxiety-like and depressive-like symptoms in the BACHD mouse model at 8 weeks old. In section 3.3.2, I established that the BACHD mice show some motor impairment, but do not exhibit differences in spontaneous activity from the WTs (Figure 3.2 and Figure 3.3). It is important to assess any evidence of motor impairments at this stage that could confound the BACHD mice's performance in the affective tests. As I did not identify a difference in spontaneous activity and minimal difficulty in rotarod, the data suggests that any differences in the following tasks at this age are a result of affective abnormalities and not a motor impairment.

I utilized the Elevated Zero Maze (EZM) and Open Field (OF) Test to measure exploration in a novel environment as a measure of anxiety-like behavior. In the EZM, exploration is measured by the latency to enter the open arm, the duration of time spent in the open arm, the number of entries into the open arm, and the number of head dips (Figure 3.5). The BACHD mice spent less time in the open arm ($t_{37} = 2.805$, $p = 0.008$), made fewer entries into the open arm ($t_{37} = 3.364$, $p = 0.0018$, and exhibited fewer head dips (t_{37}, $p < 0.0001$). This is suggestive of an anxious phenotype. The latency to enter the open arm did not significantly differ between groups ($t_{37}= 1.905$, $p = 0.065$).

In the open field test, in which exploration is measured by distance traveled in the center of an open field, and the time spent in the center, the BACHD mice did not exhibit an anxious phenotype. There was no difference in time spent in the center between groups (Fig. 3.6, $t_{22}= 1.433$, $p = 0.166$) or in the distance that was traveled exclusively in

the center of the open field (Fig. 3.6, t_{22}= 0.384, p = 0.705).

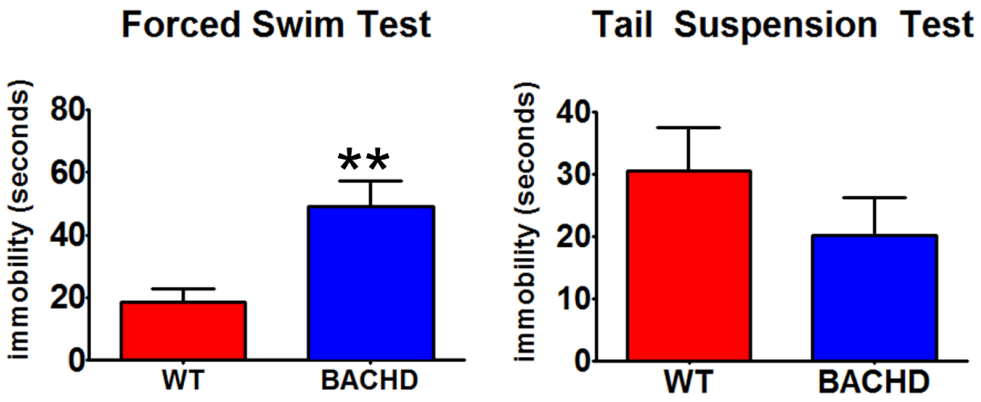

Figure 3.4: Immobility in the Forced Swim Test (FST) and Tail Suspension Test (TST). At 8 weeks old, the BACHD mice spent more time immobile in the FST and in the TST, indicating that the BACHD mice show a depressive phenotype at this age. ** p < 0.01.

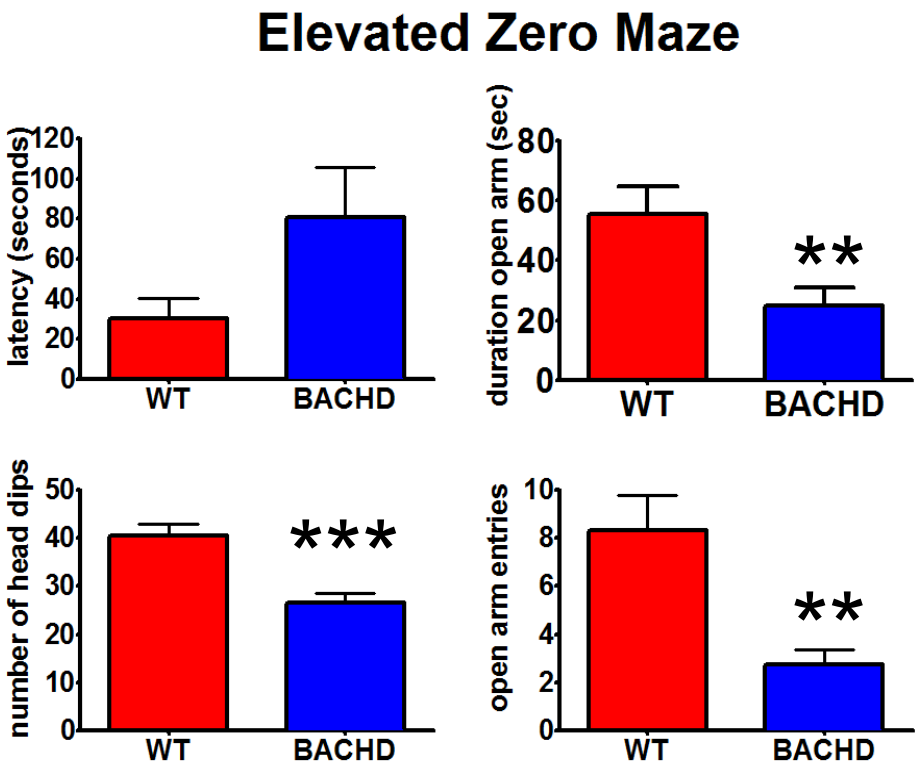

Figure 3.5: Exploration in the Elevated Zero Maze. At 8 weeks old, BACHD mice explored the novel EZM less than WT controls, as evident by less time spent in the open arm, fewer head dips, and fewer entries into the open arm by BACHD mice. **: $p < 0.01$. ***: $P < 0.001$.

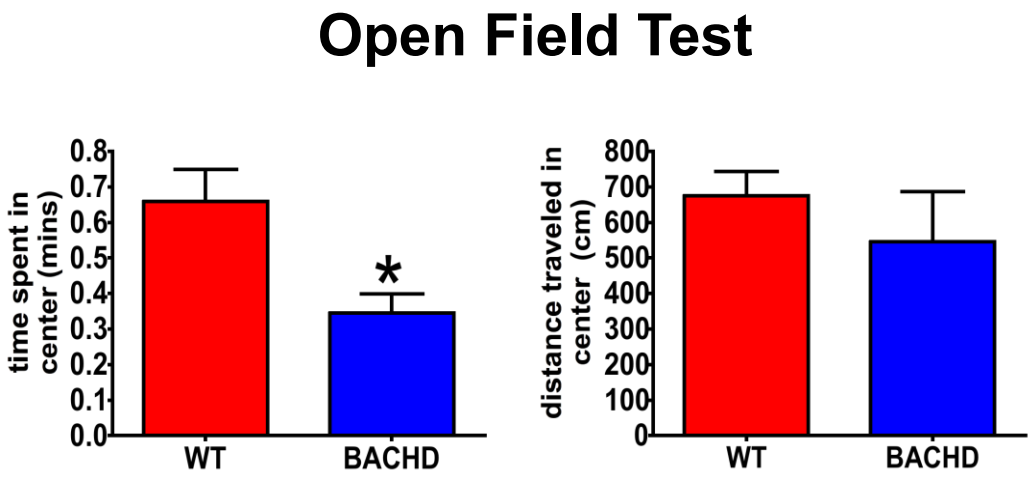

Figure 3.6: Exploration in the Open Field Test. In the OF test, BACHD mice spent significantly less time in the center during the first five minutes of the OF test. There was no difference in the distance traveled in the open field between groups, indicating that the BACHD mice were not less active in the OF test, showing that the BACHD mice did exhibit increased avoidance of the center of the OF box. *: $p < 0.05$.

I observed a similar phenomenon when testing for evidence of a depressive-like phenotype in the BACHD mice. When tested in the Forced Swim Test (FST) and the Tail Suspension Test (TST), the mice also showed evidence of a depressive phenotype in the FST. Depressive-like behavior measured as time spent immobile in the FST, which is a measure of 'behavioral despair' (Cryan et al. 2005). In the FST, the BACHD mice spent more time immobile compared to the WTs [Fig. 3.4, $t(29) = 3.321$, $p = 0.0024$]. In the TST, the time spent immobile did not differ between genotypes.

3.4 Summary of Results

To summarize, it appears that at this age point (8 weeks old), the behavior of the HD mice does reflect the prodromal stage of HD that is observed in patients. Patients, at this stage of the disease, primarily exhibit psychiatric-like symptoms of anxiety and depression with little to no motor symptoms (Thompson et al. 2011). The BACHD mice,

at the age studied, show a motor impairment on the more difficult speeds of the rotarod task but no impairments in spontaneous locomotor activity. Given the progressive deficits in motor performance the BACHD mice show in the rotarod task at 16, 20 and 24 weeks, it is evident that the BACHD mice show only a mild impairment in overall motor ability at 8 weeks old. The BACHD mice's ability to stay on the rotating rod declined with age.

When I assessed the presence of any behavioral abnormalities in the BACHD mice at the same age point, we did identify an anxiety-like and depressive-like phenotype in the BACHD mice. The anxiety-like phenotype was evident as decreased exploration in the novel EZM. Their behavior in the open field test did not differ from WTs. The depressive phenotype was evident as increased time spent immobile in the FST test. However, in the TST, the BACHD did not spent significantly less time immobile than the WTs. Typically, it is recommended to use multiple tasks to quantify behavioral abnormalities in mice in order to have strong face validity (Crawley and Paylor 1997).

3.5 Commentary

It is recommended that a battery of tests be used to fully assess the face validity of an animal model of neurodegenerative or psychiatric diseases (Sukoff Rizzo and Crawley 2017). This is, in part, because these illnesses manifest with multiple symptoms in humans. Particularly, in psychiatric illnesses, the behavioral symptoms can also vary amongst individuals and as well as rodents. Therefore, conducting a battery of tests, typically one or two tests, is a common strategy to account for this variation in behavior. I utilized this approach to investigate the face validity of the BACHD mouse model as an animal model of psychiatric illness, including anxiety and depression. I measured

depressive-like behaviors using the forced swim test and the tail suspension test. These two tests are used to measure ‘behavioral despair’ in the mice. The dependent measure of time spent immobile in the FST and TST is considered a passive coping mechanism to stressful stimuli, similar to the coping mechanisms exhibited by individuals with depression (Cryan et al. 2005). Anxiety-like behaviors were measured using the open field test and the elevated zero maze. These tests are dependent on the behavior of rodents in approach-avoidance conflict situations in which behavior is dictated by a balance between the instinct of the mice to explore a novel environment and the protective instinct to avoid open, brightly lit environments (Ramos 2008; Sukoff Rizzo and Crawley 017).

Surprisingly, the BACHD mice only exhibited a depressive-like phenotype and an anxiety-like phenotype in the FST and EZM but not in the TST and OF test respectively. The fact that the mice did not exhibit the behavioral phenotype in every test, does not rule out that the BACHD mice do exhibit the behavioral phenotype. There are potential reasons that the mice did not show anxiety-like and depressive-like phenotype in both tests used. In regards to the FST and TST, the BACHD mice did not spend more time immobile relative to the WTs. Rather, there is evidence of the BACHD spending less time immobile than the wildtype mice. One characteristic of the TST is that the bouts of immobility in this test are shorter compared to the FST, which could result in a lack of differences between the wildtype and BACHD mice (Cryan et al. 2005). Surprisingly, the BACHD mice spent less time immobile compared to wildtypes. It has been shown in other mouse models of depression that intra-species differences in baseline TST and FST activity occur. A study by Bai and colleagues (2001) observed that the NIH-Swiss mice

showed less immobility in the TST than the FST. One possible explanation is that the FST is considered more stressful than the TST, which can increase immobility in the FST relative to the TST (Sanchez 1997).

In the same study by Bai et al. (2001), there were inter-strain differences in the response to the tricyclic antidepressant, imipramine, in the FST and TST. In the FST, imipramine reduced immobility at lower doses and increased immobility in at higher doses in the C57BL/6 strain. On the contrary, imipramine reduced immobility in the TST at the same doses, including the higher doses. This difference in response to the same antidepressant in different tests suggests that there is a difference in the neurobiological mechanisms of the FST and TST. Imipramine elicits its effect by increasing activity of serotonin and norepinephrine by inhibiting reuptake.. However, imipramine also acts on other neurotransmitter systems, so it is unknown if the antidepressant effect of the tricyclic antidepressant in the FST and TST is dependent entirely on restoring monoaminergic activity. Interestingly, another study suggested that immobility in the TST is not associated with changes in monoaminergic activity. Renard and colleagues (2003) observed that the FST altered whole-brain tissue concentrations of dopamine and serotonin, whereas this tissue concentration was unaffected in mice following the TST, providing further evidence of a difference in the neurotransmitter systems involved in the TST. Even though imipramine acts on the main monoamines in the brain, it also acts on other neurotransmitters such as acetylcholine, and histamine. It is possible that immobility in TST is dependent on neurotransmitters outside of monoaminergic systems, but this requires further investigation.

In regards to the Open Field Test and the Elevated Zero Maze, there is evidence of differences in usefulness of each test to accurately measure anxiety-related behaviors. Carola and colleagues (2002) compared the performance of C57BL/6 and BALB/c in the open field test and in the elevated plus maze (EPM; similar to the elevated zero maze). The study showed that the C57BL/6 mice were equally active in both the OF test and EPM, but that the mice restricted all movement to the closed arm of the EPM. Whereas the mice traveled in the center and periphery of the OF test and did not show avoidance of the center of the OF arena. This suggests that the open arm of the EPM arena was more aversive to the mice than the center of the OF, supporting that the EPM is more anxiogenic than the OF test. I observed a similar phenomenon in which the BACHD mice did not show a preference for the center of the OF test and showed active rearing in the closed arm of the EZM (data not shown). Therefore, it is likely that the OF test and the TST are not sensitive enough to detect differences in the anxiety-related and depressive-related behavior in the BACHD mice. In the future, other tests besides the TST and OF test are recommended to measure anxious behaviors of the BACHD mice.

CHAPTER IV:

Reduced serotonergic activity in the limbic ventral hippocampus in BACHD mice: association with affective disorder

4.1 Rationale:

In Chapter 3, it was established that symptoms in the BACHD mouse model emerge in stages, similar to the human condition. The stage that I was primarily interested in was the early prodromal, or non-motor stage of the disease, in which patients exhibit psychiatric and cognitive symptoms and little to no motor difficulties. I identified that the BACHD mice show a similar behavioral phenotype at 8 weeks old, with evident anxious and depressive symptoms concurrent with mild motor symptoms (see section 3.3.2 and 3.3.3). This supports the use of the BACHD mouse model as a candidate for investigating the pathophysiology of the symptoms of the early prodromal stage of HD. Whilst I determined that the BACHD mouse model exhibits face validity of the prodromal stage of HD, it remains unknown if this mouse model meets the requirement of construct validity of the early non-motor phenotypic stage of HD. In this chapter, I present subsequent experiments in the BACHD mouse model that examined the construct validity of this model for the equivalent prodromal stage of HD.

The criterion for construct validity is that the mouse model must show a similar pathological change related to the behavioral symptoms in humans and the animal model (Feusner et al. 2009). From clinical studies, it is hypothesized that the affective disorder in HD is the result of a deficit in serotonergic neurotransmission, which can be alleviated by selective serotonin reuptake inhibitors that increase and facilitate serotonin signaling (Rowe et al. 2012). This implies that reduced serotonin activity is due to increased reuptake by the serotonin transporter. However, there is no evidence that the serotonin transporter is affected in HD patients (Pla et al. 2014). Instead, this phenomenon suggests

that disruptions in other aspects of serotonergic signaling can manifest in affective disorders, which may be remediated by the increased synaptic availability of serotonin. This is supported by the fact that the therapeutic effect of SSRIs is delayed until the 5-HT1A autoreceptor expression in the raphe is downregulated, highlighting that the mechanism of action of these drugs is through adaptive changes in serotonin neurotransmission in the brain (Blier and Montigny 1994; Delgado 2004). Given this uncertainty, it was difficult to assess the construct validity of the psychiatric symptoms in the HD mice. Rather, it was beneficial to use the mouse models to identify potential mechanisms underlying the mood abnormalities that could explain these symptoms in HD patients. This investigation was largely informed by studies of idiopathic depression and anxiety, which identified several potential impairments in serotonergic function that is thought to underlie the neurobiology of these symptoms.

Serotonergic signaling can be disrupted through several mechanisms, including altered reuptake via the serotonin transporter, impaired neuronal firing, altered metabolism due to dysfunction of the monoamine oxidase enzyme, and dysfunction at any of the 14 serotonin receptor subtypes in the brain (Lesch et al. 2003). In the incidence of affective disorders, common deficits in serotonin activity have been attributed to increased metabolism by monoamine oxidase, reduced 5-HT1A/5-HT1B receptor expression and binding, increased 5-HT2C receptor activity, as well as altered serotonin transporter function and expression in patients with Major Depressive Disorder and Generalized Affective Disorder (Stockmeier 2003; Bhagwagar et al. 2004; Bhagwagar et al. 2006; Bhagwagar et al. 2007; Meyer et al. 2006; Drevets et al. 2007; Munafo et al. 2008). Of those features, impaired status of the 5-HT1A receptor (Cross et al. 1986;

Waeber and Palacios 1989) and increased metabolism of serotonin by monoamine oxidase was implicated in HD patients (Richards et al. 2011). Importantly, this indicates that serotonin activity can be disrupted by means of several mechanisms to manifest in anxiety and depression. This is an important distinction that implicates serotonin in affective disorders and reveals the complexity of the involvement of serotonin in affect that is not explained by the monoamine hypothesis of affective disorders.

It has been shown in other HD mouse models that tissue levels of serotonin are reduced in the cortex and hippocampus in the R6/1 and R6/2 mouse model (Pang et al. 2009; Renoir et al. 2011; Pouladi et al. 2012). However, this approach cannot be used to address the multiple mechanisms of serotonergic signaling that are impaired in affective disorders. Using a different approach – by measuring serotonin efflux – I was able to assess the synaptic availability of serotonin, in limbic structures in the HD mice. Importantly, the benefit to measuring serotonin efflux over tissue concentrations of serotonin is the added ability to examine changes in serotonin efflux temporally and the ability to examine deficits in different processes of neurotransmission that yields serotonin efflux. Serotonin efflux is a function of serotonin release, reuptake, receptor binding and diffusion (Kirby and Lucki 1995).

Therefore, I measured *in vivo* serotonin efflux to observe if there are impairments in serotonergic neurotransmission in the BACHD mice. Raphe serotonergic neurons project to several limbic structures, including the prefrontal cortex, amygdala, ventral hippocampus, and ventral striatum. Of particular interest is the serotonergic input to the ventral hippocampus. Serotonin regulates the response to anxiogenic and stressful stimuli

via this projection by increasing and reducing serotonin efflux respectively in the vHPC (Kirby and Lucki 1995; Amat et al. 1998; Kagamiishi et al. 2003). This is thought to elicit behavioral responses by regulating communication between the vHPC and amygdala to signal approach or avoidance behaviors in response to the anxious or stressful stimuli (Lowry and Hale 2010, in *Handbook of Behavioral Neurobiology of Serotonin*). However, it is not known if a reduction or increase in serotonin activity in the vHPC results in anxious and depressive behaviors. Therefore, it is useful to measure serotonin efflux in the vHPC of a mouse model of comorbid anxiety and depression such as the BACHD mice. Not only will this provide insight into the impairments in serotonergic neurotransmission that drives the psychiatric symptoms in HD, it will also provide insight into the etiology of idiopathic affective disorders.

Additionally, I measured serotonin and dopamine efflux in the dorsal striatum. This was done to investigate if dopamine efflux was altered in the dorsal striatum, which could be suggestive of motor impairments, as it has been shown previously in other HD mouse models (Callahan and Abercrombie 2011). I also measured serotonin efflux in the striatum to see if serotoninergic activity outside of the limbic system was affected.

4.2 Brief Overview of Methods:

The microdialysis experiments were conducted after the completion of all behavioral tasks. Two different experimental designs were used. In one cohort, a microdialysis probe was implanted in the ventral hippocampus (AP: -2.5, ML ± 3, DV: -4), to measure serotonin efflux *in vivo*. In a separate cohort of mice, two dialysis probes were implanted, with the objective to measure dopamine and serotonin activity *in vivo*.

One probe was implanted in the dorsal striatum (AP: +0.75, ML: ± 2, DV: -4), and one probe was implanted in the contralateral ventral hippocampus (AP: -2.5, ML ± 3, DV: -4). Approximately 16-18 hours after the surgery, 10-15 baseline dialysate samples were collected and frozen at -80°C until quantification. Basal extracellular serotonin and dopamine levels were measured and quantified using High Performance Liquid Chromatography (HPLC) with electrochemical (EC) detection. The average baseline level of the monoamines was determined from 3 samples with levels within 10-15% variability. The serotonin metabolite, 5HIAA and the dopamine metabolite, DOPAC, were also quantified from 3-5 samples with 10-15% variability.

4.3 Results

Table 4.1 depicts the average age at the point of microdialysis experiments, which ranges from 73.00 days ± 8 (10 weeks old) to 80.20 days ± 4.82 (for striatal dopamine measures and 85.00 days ± 3.42 to 87.77 days ±3.82 for hippocampal serotonin measures. Both experiments were conducted at approximately 10-12 weeks old. Therefore, the mice were approximately 2-4 weeks older at the time of microdialysis experiments compared to the age of all behavioral experiment

Age of mice (days) during microdialysis experiments in striatum and vHPC				
	Monoamines and Metabolites in Striatum		Serotonin and Metabolites in vHPC	
	WT	BACHD	WT	BACHD
Age	73.00 ± 8.00	80.20 ± 4.82	85.00 ± 3.42	87.77 ± 3.82
N	4	7	15	15

Table 4.1: Approximate age and number of WT and BACHD mice included in microdialysis experiments. All testing in microdialysis experiments was conducted after the conclusion of all behavioral tests, which resulted in all mice being tested at the approximate age of 73 to 88 days old (10-12 weeks old). This is the total number of mice with confirmed correct probe placement in the targeted regions.

4.3.1 *In Vivo* detection of dopamine and serotonin in the BACHD mice

Serotonin and dopamine are implicated in motivation, reward, and affective behaviors. Conversely, abnormalities in dopamine and serotonin signaling are implicated in affective disorders (Chaudhury et al. 2015). Therefore, I wanted to address if dopamine and serotonin activity were impaired in the symptomatic BACHD mice. To examine changes in monoaminergic activity in the symptomatic BACHD mice, I measured dopamine and serotonin efflux (using *in vivo* microdialysis) in the dorsal striatum, a region involved in motor ability and motivation (Ikemoto et al. 2015). Dopamine and

serotonin efflux were unaffected in the dorsal striatum [Fig. 4.2, DA: t (8) = 1.167, p = 0.277; 5-HT: t (9) = 0.023, p = 0.982 respectively]. Similarly, the concentration of DOPAC and 5HIAA in the dorsal striatum was similar between WTs and BACHD mice [Fig 4.2; DOPAC: t (9) = 0.789, p = 0.451; 5HIAA: t (9) = 0.120, p = 0.908). I also measured serotonin efflux in the ventral hippocampus, a region involved in regulating limbic system function (Hensler et al. 2006). Serotonin efflux was reduced in the ventral hippocampus of BACHD mice compared to wild-types (Fig. 4.1; t (34) = 2.971, p = 0.0054). Furthermore, I measured the amount of the serotonin metabolite, 5-hydroxyindoleacetic acid (5HIAA) and the dopamine metabolite, 3,4-Dihydroxyphenylacetic acid (DOPAC) in the vHPC. The amount of 5HIAA [Fig. 4.1 t (15) = 0.828, p = 0.421] and DOPAC [Fig. 4.2, t (15) = 0.324, p = 0.750] in the ventral hippocampus was not significantly different between wild-types and the transgenic mice.

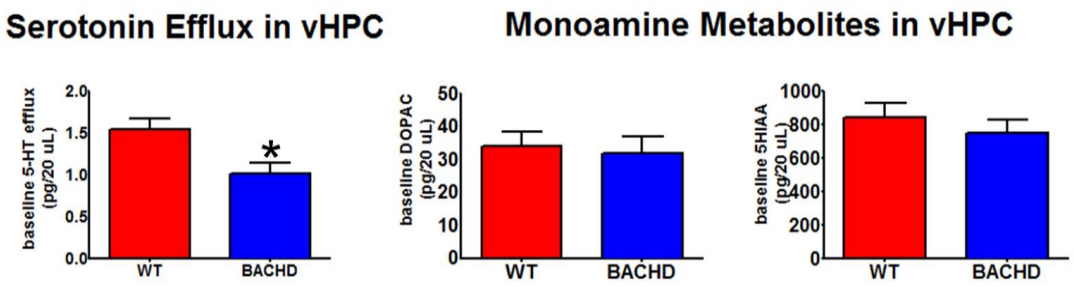

Figure 4.1: Serotonin efflux, DOPAC and 5HIAA levels in the ventral hippocampus of WT and BACHD mice. In the early disease state of HD in this mouse model (10-12 weeks old), the BACHD mice have reduced serotonin efflux in the ventral hippocampus. DOPAC and 5HIAA levels are unaffected at this age. *: $p < 0.05$.

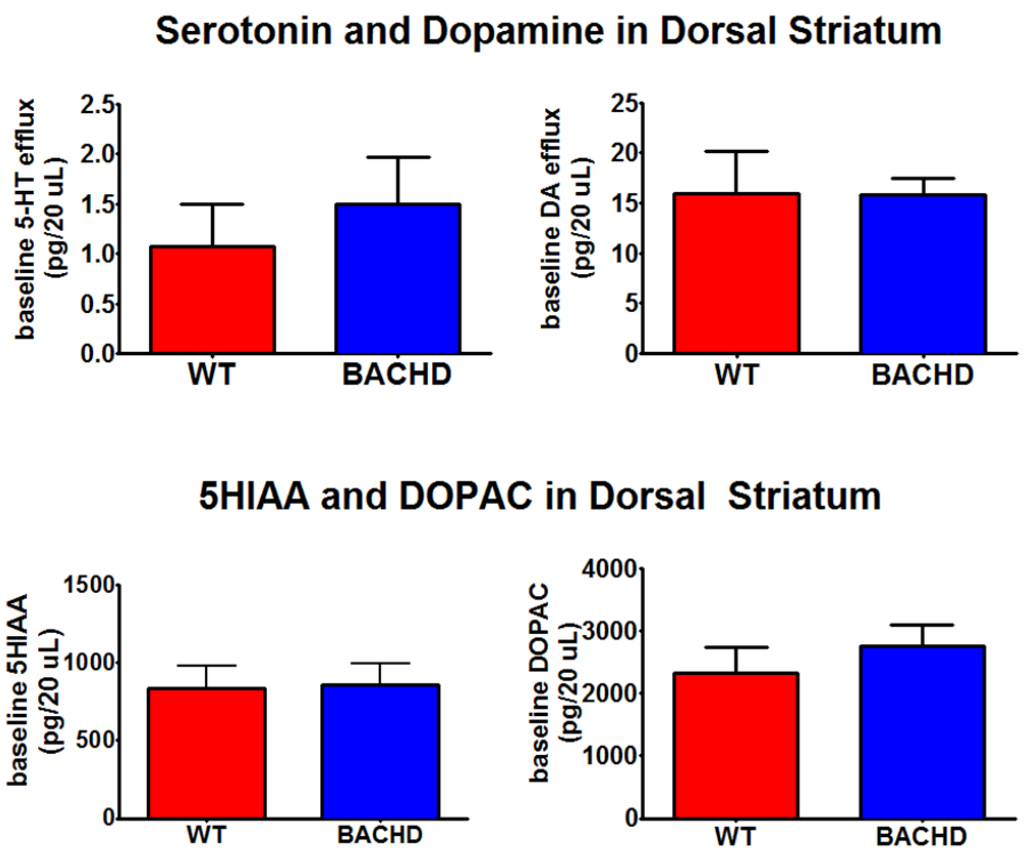

Figure 4.2: Dopamine and serotonin efflux, DOPAC and 5HIAA levels in the dorsal striatum of WT and BACHD mice. In the early stage of HD in this mouse model (10-12 weeks old), serotonin efflux in the dorsal striatum is similar between groups. Dopamine efflux is slightly decreased in the BACHD mice, though this is not significant.

4.3.2 Association between extracellular 5-HT and affective behavior

The association between serotonin efflux and anxiety and depression in an animal model is not clear in the existing literature as few studies have examined if there is a correlation between serotonin levels i*n vivo* and any abnormalities in behavior. Therefore, I analyzed the data to see if there is an association between serotonin efflux and anxiety-like/depressive-like symptoms. I examined this relationship between serotonin efflux and

activity in the EZM and FST as there were significant differences between the WTs and BACHD performance. I did not assess any correlation between behavior in the OF test and TST, as the results were not significantly different between groups. Furthermore, in the EZM, I only analyzed the behavioral measures that were significantly different between genotype groups. This included the duration of time spent in the open arm, the number of open arm entries, and the number of head dips. The latency to enter the open arm was excluded because it was not significantly different. To analyze the correlations, I plotted the behavioral measures and serotonin efflux. The data for the WTs and BACHD mice were analyzed separately. In the FST, there were no significant correlations between the time spent immobile and serotonin efflux in vHPC for either the WTs (Fig.4.3, Pearson's $r = 0.413$, $p = 0.126$) or BACHD mice (Fig. 4.3, Pearson's $r = -0.273$, $p = 0.325$). Even though the correlation was not significant, there appears to be a positive trend in the WT behavior as related to serotonin efflux in the vHPC. Animals with higher levels of serotonin efflux spent more time immobile in the FST. However, in the BACHD mice, there is no clear association between the efflux levels and the behavioral measure in the FST.

I also analyzed the correlation between exploratory behaviors in the EZM and serotonin efflux. There was no significant correlation between the time spent in the open arm of the EZM and serotonin efflux in the vHPC for either WTs (Fig. 4.4, Pearson's $r = -0.180$, $p = 0.521$) or the BACHD mice (Fig. 4.4, Pearson's $r = 0.188$, $p = 0.503$). The same result was observed for the number of open entries (WTs: Fig. 4.4, Pearson's $r = -0.041$, $p = 0.884$; BACHD mice: Fig. 4.4, Pearson's $r = 0.230$, $p = 0.332$) and the number

of head dips (WTs: Fig. 4.4, Pearson's r = -0.152, p = 0.589; BACHD mice: Fig. 4.4, Pearson's r = 0.360, p = 0.187).

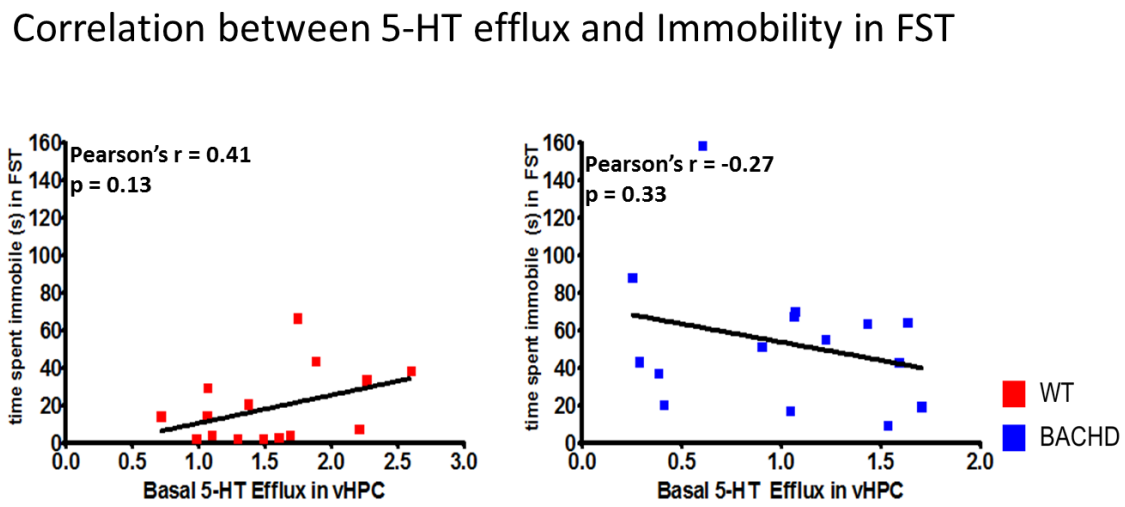

Figure 4.3: Correlation between extracellular serotonin efflux in vHPC and immobility in the FST. There was no significant correlation between serotonin efflux and the time spent immobile in the FST in the BACHD mice or in WTs.

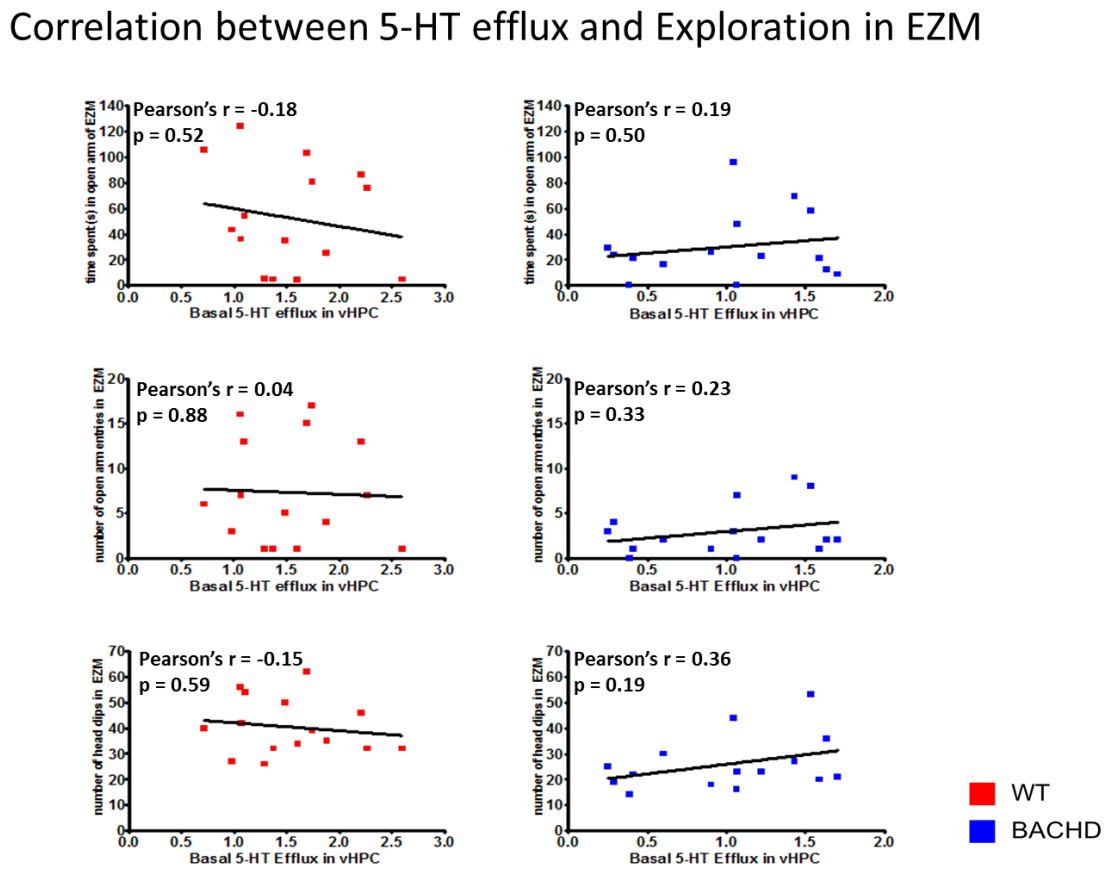

Figure 4.4: Correlation between extracellular serotonin efflux in vHPC and exploratory behavior in the EZM. There was no significant correlation between serotonin efflux and the time spent in the open arm of the EZM, the number of entries into the open arm, and the head number of head dips in the EZM in the BACHD mice or in WTs.

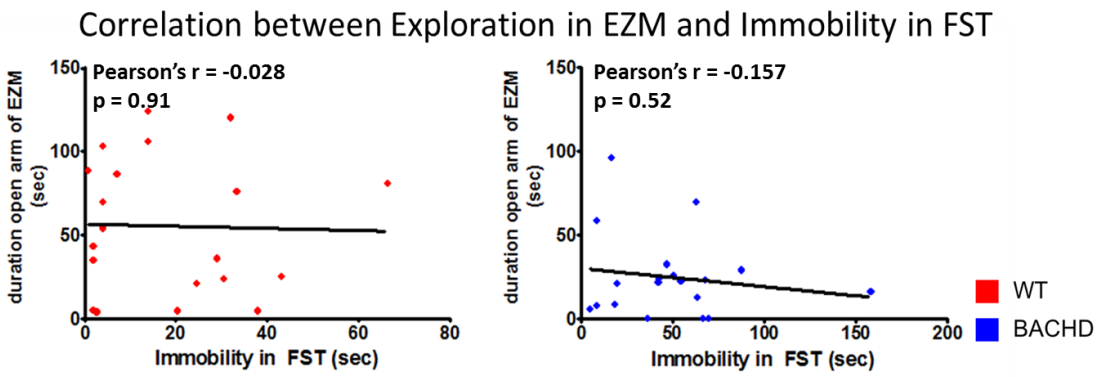

Figure 4.5: Correlation between exploration in EZM and immobility in FST. There was no significant correlation between the time spent in the open arm of the EZM and the time spent immobile in FST in either WT or BACHD mice.

Lastly, I analyzed the evidence of a correlation between the depressive-like measure in the FST and the anxiety-like measure in the EZM (Figure 4.5). There was no significant correlation between immobility in the FST and time spent in the open arm of the EZM in either the WT (Pearson's r = -0.028, p = 0.91) or BACHD (Pearson's r = -0/157, p = 0.52) mice.

4.3.3 Confirmation of probe placement in striatum and ventral hippocampus

Following the completion of microdialysis experiments, all mice were sacrificed by a lethal injection of chloral hydrate and perfused with a 4% paraformaldehyde solution containing 15% picric acid. Brains were removed and stored in fixative overnight at 4°C. Brains were then placed in a 30% sucrose solution until sectioned. Brains were sectioned using a freezing microtome and cut into 60 micron sections. Sections were mounted onto gelatin-subbed slides, dried overnight and stained with a neutral red solution to visualize the probe track in the ventral hippocampus or dorsal striatum. Figure 4.5 shows an example of a histological section containing a probe track in the ventral hippocampus. All data shown here were taken from animals in which the probe placement was confirmed in

the ventral hippocampus or dorsal striatum (vHPC: 15 WT and 15 BACHD mice; dorsal striatum: 4 WT and 7 BACHD mice).

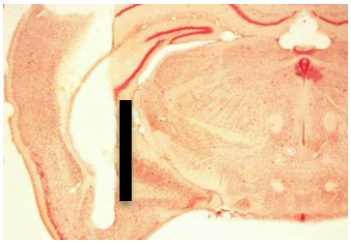

Figure 4.6: Photomicrograph of Neutral-Red stained histological section demonstrating representative probe placement in ventral hippocampus. The active zone is a 2 mm area depicted by the black bar.

4.4 Summary of results

The purpose of the experiments detailed in this chapter was to identify potential neural correlates of the anxious and depressive phenotype exhibited by the BACHD mice. Additionally, I assessed serotonergic and dopaminergic activity in the dorsal striatum to see if serotonin and dopamine function outside the limbic system was affected. In the dorsal striatum, dopamine and serotonin levels were similar in the WTs and BACHD mice. Furthermore, I measured the quantity of the neurotransmitter metabolites (DOPAC and 5HIAA) in the striatum, which were also similar between groups. The dorsal striatum is a region primarily involved in voluntary motor behavior and action selection (Cui et al. 2013). I measured monoamines in this structure to observe if there were any neurochemical impairments that could explain the motor deficits or the observed affective symptoms. As dopamine and serotonin function were intact in this brain region, this further solidifies that this age in the HD mice is representative of the

prodromal stage, in which motor symptoms and the accompanying brain regions involved in that behavior remains mostly intact.

In addition to measuring monoamine levels in the dorsal striatum, I measured serotonin efflux and the metabolites, DOPAC and 5HIAA, in the ventral hippocampus (vHPC). I measured this in vHPC because it is part of a brain circuit that functions to regulate mood and behavior (Padilla-Coreano et al. 2016). I did not measure dopamine in the vHPC because the projections from dopaminergic nuclei to this brain region are sparse and dopamine is rapidly metabolized in the vHPC, so any levels detected were very low. Instead, the amount of DOPAC can reflect dopaminergic activity in this region. The levels of the metabolites, DOPAC and 5HIAA, did not differ between the WTs and BACHD mice. Serotonin efflux was decreased by approximately 30% in the BACHD mice. Decreased serotonin efflux could potentially account for the observed behavioral results though this was not significant. This was investigated in additional experiments to be discussed in the next chapters.

In vivo serotonin efflux was measured in the same mice that were tested behaviorally in the affective tests. The measured affective behaviors were compared to the amount of serotonin efflux in vHPC to see if there was a correlation between serotonin efflux and anxiety or depressive symptoms. I only analyzed the behavioral measures that were statistically significant. Therefore, the time spent immobile in the FST, the duration of time spent in the open arms of the EZM, the number of open arm entries in EZM, and the number of head dips in the EZM was compared to serotonin efflux in vHPC. This was analyzed separately for the WTs and BACHD mice. No

significant correlations between serotonin efflux and behavioral measures were found for any of the 4 tests, for both WTs and the BACHD mice.

CHAPTER V:

Treatment of affective phenotype in BACHD mice with selective serotonin reuptake inhibitor, escitalopram and 5-HT1A agonist, 8-OH-DPAT

5.1 Rationale:

The final criterion for assessing the validity of the BACHD mouse model for the investigation of affective disorders in HD is predictive validity. Predictive validity dictates that the translational mouse model responds similarly to the same identified treatments for the symptoms in patients (Feusner et al. 2009). In addition to investigating the predictive validity of common pharmacological treatment of affective disorder in the BACHD mice, I was also interested in identifying novel treatments for these behavioral abnormalities.

The onset of anxiety-like and depressive-like symptoms in the BACHD mouse model is present concomitantly with impaired serotonin signaling in the vHPC, a limbic structure hypothesized to contribute to the incidence of anxiety and depression (Amat et al. 1998; Kagamiishi et al. 2003, Kirby and Lucki 1995; Lowry and Hale 2010). However, there was no significant correlation between serotonin efflux in the vHPC and the anxious or depressive behaviors exhibited by the BACHD mice (see section 4.3.2). This suggests that the synaptic availability of serotonin (detected as extracellular serotonin levels) does not directly underlie the deficits in affective behavior exhibited by the BACHD mice.

Extracellular serotonin is determined by the balance between serotonin release, reuptake, metabolism, diffusion and receptor binding in proximity of numerous synaptic clefts (Kirby and Lucki 1995). It has been shown that impairments in each one of those mechanisms can result in the manifestation of anxious or depressive symptoms. In the incidence of affective disorders, common deficits in serotonin activity have been

attributed to increased metabolism by monoamine oxidase, reduced 5-HT1A/5-HT1B receptor expression and binding, increased 5-HT2C receptor activity, as well as altered serotonin transporter function (Stockmeier 2003; Bhagwagar et al. 2004; Bhagwagar et al. 2006; Bhagwagar et al. 2007; Meyer et al. 2006; Drevets et al. 2007; Munafo et al. 2008). Those are all potential therapeutic targets for both existing and for novel antidepressants and anxiolytics.

In HD patients, anxiety and depression is typically treated with selective serotonin reuptake inhibitors (Rowe et al. 2012), but has been successfully treated with monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), benzodiazepines, antipsychotics that are primarily dopamine D2 receptor antagonists, selective norepinephrine reuptake inhibitors, and in a few cases, 5HT2 receptor antagonists (Anderson and Marder 2001). This diversity of treatment suggests that the psychiatric symptoms of HD may be a factor of deficits in signaling of multiple neurotransmitter systems such as dopamine and norepinephrine. Therefore, the use of the SSRIs should be considered a nonselective treatment that does not directly target the impairments in neurotransmission that likely occurs in HD patients.

For this reason, I was interested in comparing the therapeutic efficacy of SSRIs with another class of drugs that selectively restores and mimics serotonin activity, 5-HT1A agonists. 5-HT1A agonists were shown in preclinical and clinical studies to have antidepressant and anxiolytic properties (Lucki et al. 1994; Fang et al. 2011). Dysfunction of serotonin receptors is frequently implicated in anxiety and depression; particularly the 5-HT1A, 5-HT1B, and 5-HT2A receptor (see reviews Gingrich and Hen

2001; Lesch et al. 2003; Nautiyal and Hen 2017). Patients with MDD are found to have increased expression of the 5-HT1A receptor as revealed through postmortem and position emission tomography (PET) imaging studies (Stockmeier et al. 1998; Kaufman et al. 2015). 5-HT1A receptor knockout mice showed decreased depressive behavior and increased anxiety-like behavior (de Vry 1995; Heisler et al. 1998; Parks et al. 1998; Ramboz et al. 1998). In humans, allelic variations in the 5-HT1A receptor were associated were more anxious personalities, reduced 5-HT1A receptor density in cortical regions were found in patients with higher levels of anxiety (Tauscher et al. 2001; Strobel et al. 2003). In the HD mouse models, there was evidence of diminished 5-HT1A, 5-HT1B, and 5-HT2A serotonin receptor expression (Pang et al. 2009; Renoir et al. 2010) and impaired 5-HT1A receptor binding (Renoir et al. 2013), as well reduced binding at 5-HT1A receptor sites in HD patients postmortem (Cross et al. 1986; Waeber and Palacios 1989). Therefore, the 5-HT1A receptor was a potential postsynaptic target for novel therapeutics.

In brief, the experiments in this chapter were designed to test the validity of the common antidepressant/anxiolytics and novel therapeutics. The behavioral and neurochemical response to escitalopram and 8-OH-DPAT were investigated for the ability to ameliorate the psychiatric-like symptoms in the BACHD mouse model. This was assessed by testing the anxiolytic and antidepressant properties of both drugs in the EZM and FST. Additionally, I investigated if the therapeutic action of these drugs was dependent on increasing serotonin activity *in vivo* by measuring serotonin efflux in the ventral hippocampus.

5.2 Brief Overview of Methods:

The primary experiments in this chapter investigated the efficacy of the selective serotonin reuptake inhibitor to alleviate both the behavioral and pathological impairments in the BACHD mouse model that is the suspected etiology of the affective disorder in HD.

5.2.1 Anxiolytic and antidepressant properties of escitalopram and 8-OH-DPAT

The BACHD mice exhibited anxiety-like behavior in the EZM and depressive-like symptoms in the FST. The EZM and FST are common tests used to screen anxiolytic and antidepressant properties of pharmacological agents. Subsequently, I used these tests to screen two classes of drugs for the potential to alleviate the affective disorders exhibited by the BACHD mice. The first class includes the SSRI escitalopram. The second class of drugs is a serotonin 1A receptor agonist, 8-OH-DPAT.

Escitalopram was tested at two doses in the EZM and FST, 0.1 mg/kg and 1.0 mg/kg. The drug was dissolved in saline and injected intraperitoneally at a volume of 10 mL/kg. Escitalopram was administered 60 minutes prior to testing for both tests as the peak concentration of escitalopram in the brain and the maximal increase in serotonin following administration is reached 1 hour post-injection (Kreilgaard et al. 2008; Nguyen et al. 2012). 8-OH-DPAT was tested at two doses, 0.3 mg/kg and 3.0 mg/kg. The drug was dissolved in saline and injected subcutaneously at a volume of 10 mL/kg. 8-OH-DPAT was administered 30 minutes prior to testing as the maximal effect of the drug on

serotonin occurs within 30 minutes of systemic administration (Yu et al. 1996). The EZM and FST tests were administered in the same procedure as described in chapter 1. The EZM test lasted five minutes; the trial was videotaped and scored later for latency to enter the open arm, duration in the open arm, number of open arm entries, and the number of head dips. The FST lasted six minutes, was videotaped and scored for time spent immobile.

5.2.2 Treatment of reduced serotonin efflux in vHPC of BACHD mice and wildtype controls

The neurochemical response to escitalopram and 8-OH-DPAT was measured in the WT and BACHD mice following the completion of behavioral testing. Separate cohorts of WT and BACHD mice were tested in this experiment. Some cohorts were tested behaviorally in the FST and EZM in absence of pharmacological testing (baseline measure of anxiety-like and depressive-like behavior), which mean these cohorts only received drug treatment once. Other cohorts were tested behaviorally in the FST or EZM following administration of vehicle, 8-OH-DPAT or escitalopram, and would have received drugs twice, once in behavioral tests and once in microdialysis experiments.

The neurochemical response to 8-OH-DPAT and escitalopram was assessed by measuring sernin efflux in the ventral hippocampus. Each drug was administered following the collection of 10 baseline dialysate samples. 8-OH-DPAT was administered systemically via a subcutaneous injection. 8-OH-DPAT was dissolved in saline and injected in a volume of 10 mL/kg. Serotonin efflux was measured two hours post-injection of 8-OH-DPAT. Escitalopram was administered locally via reverse dialysis

through the microdialysis probe into the ventral hippocampus. Escitalopram was dissolved in artificial cerebrospinal fluid (aCSF) in three concentrations 0.1 μM, 1.0 μM, and 10.0 μM. Each dose of escitalopram was infused for one hour locally during which dialysate samples were collected simultaneously and for one hour post infusion. The drug was infused at a rate of 1.5 μl/min. During the post infusion, aCSF without escitalopram was administered to wash out the drug. Each mouse only received one treatment, either 8-OH-DPAT or escitalopram.

5.3 Results

A total of 20 WT and 20 BACHD mice were utilized to test the therapeutic efficacy of the SSRI, escitalopram and the 5-HT1A agonist, for the treatment of anxious-like and depressive-like behaviors in the EZM and FST respectively. WT and BACHD mice were 8 weeks old at the time of testing in the EZM and 9 weeks old when tested in the FST (shown in Table 5.1). The FST was administered 5-8 days after the EZM test to allow time for drug clearance from the previous administration. The mice were tested second in the FST because this test is more stressful than the EZM and could have confounded performance in the EZM. The mice were only tested on EZM and FST once, as the behavioral measures in these tasks are dependent on the novelty of the test. Therefore, this experiment was conducted using a between-subjects design with mice receiving vehicle or a dose of either 8-OH-DPAT or escitalopram. The number of mice per experimental group (vehicle or drug, and per dose) is shown in Table 5.2.

Additionally, the WT and BACHD mice that were included in the microdialysis experiments detailed in Chapter 4 were the same cohorts of mice used the microdialysis

experiments in this Chapter. The purpose of the microdialysis experiments in this chapter was to observe the pharmacological effect of escitalopram and 8-OH-DPAT on serotonin efflux in the ventral hippocampus. The age of the mice at the time of the microdialysis experiments is shown in Table 5.3.

Age of mice (days) during behavioral testing in affective tests				
	Elevated Zero Maze		Forced Swim Test	
	WT	BACHD	WT	BACHD
Age (days)	54.65 ± 1.33	55.05 ± 1.30	62.35 ± 1.38	62.05 ± 1.39
N	20	20	20	20

Table 5.1 Age of WT and BACHD mice at time of pharmacological intervention in affective tests. The average age of WT and BACHD mice at the time of pharmacological testing in the EZM and FST. WT and BACHD mice were approximately 55 days old or 8 weeks old at the time of testing in the EZM. Both WT mice and BACHD mice were tested in the EZM and FST. WT and BACHD mice were approximately 62 days or 9 weeks old at time of testing in the FST

Number of mice in affective tests following drug treatment		
Treatment	WT	BACHD
Vehicle	4	4
0.3 mg/kg 8-OH-DPAT	4	4
3.0 mg/kg 8-OH-DPAT	4	4
0.1 mg/kg escitalopram	2	2
1.0 mg/kg escitalopram	3	3

Table 5.2 Number of WT and BACHD mice used in affective tests with pharmacological intervention. The number of WT and BACHD mice tested with vehicle, 0.3 mg/kg 8-OH-DPAT, 3.0 mg/kg 8-OH-DPAT, 0.1 mg/kg escitalopram and 1.0 mg/kg escitalopram in the EZM and FST.

	Age of mice (days) in microdialysis experiments following drug treatment in vHPC	
	WT	BACHD
Age	88.20 ± 6.32	85.39 ± 3.76
N	14	13

Table 5.3 Age and number of total WT and BACHD mice used in microdialysis experiments with pharmacological treatment. The average age of WT and BACHD mice at the time of microdialysis experiments to measure the neurochemical response to varying doses of 8-OH-DPAT and escitalopram in the ventral hippocampus. WT and BACHD mice were approximately 85-88 days old or 11 weeks old at the time of testing.

These microdialysis experiments were completed in WT and BACHD after the completion of behavioral tests, which is why they are 3 weeks old than the target age of 8 weeks when the BACHD mice exhibit the psychiatric-like phenotype.

Number of mice in microdialysis experiments following drug treatment		
Treatment	WT	BACHD
0.3 mg/kg 8-OH-DPAT	2	2
3.0 mg/kg 8-OH-DPAT	2	2
0.1 μM escitalopram	4	4
1.0 μM escitalopram	6	5
10.0 μM escitalopram	4	4

Table 5.4 Number of WT and BACHD in each experimental treatment group for microdialysis experiments. The number of WT and BACHD mice tested with vehicle, 0.3 mg/kg 8-OH-DPAT, 3.0 mg/kg 8-OH-DPAT, 0.1 mg/kg escitalopram and 1.0 mg/kg escitalopram in microdialysis experiments.

5.3.1 Antidepressant properties of 8-OH-DPAT and escitalopram in the BACHD mice

A 2-way ANOVA was conducted to analyze the effect of vehicle, and either 8-OH-DPAT and escitalopram treatment on the time spent immobile in the FST. For treatment with escitalopram, 2-way ANOVA revealed no significant effect of Treatment (Figure 5.1; $F_{2, 12} = 2.099$, p = 0.0.165), Genotype ($F_{1, 12} =0.005$, p = 0.942) or Interaction effect ($F_{2, 12} = 1.670$, p = 0.229). For 8-OH-DPAT treatment, 2-way ANOVA revealed no

significant effect of Treatment (Figure 5.2; $F_{2, 17} = 2.174$, p = 0.0.144), Genotype ($F_{1, 17} = 1.876$, p = 0.189) or Interaction effect ($F_{2, 17} = 2.293$, p = 0.131).

5.3.2 8-OH-DPAT but not escitalopram had an anxiolytic effect in the BACHD mice

The anxiolytic properties of 8-OH-DPAT and escitalopram was determined by comparing the duration the WT and BACHD mice spent in the open arm of the EZM after treatment with vehicle, 8-OH-DPAT or escitalopram. For time spent in the open arm of the EZM, there was no significant effect of escitalopram Treatment (Figure 5.1; $F_{2, 12} = 1.723$, p = 0.220), Genotype ($F_{1, 10} = 3.705$, p = 0.0.078) or Interaction effect ($F_{2, 12} = 0.526$, p = 0.604).

For time spent in the open arm of the EZM, there was a significant effect of 8-OH-DPAT Treatment (Figure 5,2; $F_{2, 18} = 3.252$, p = 0.042), but no significant effect of Genotype ($F_{1, 18} = 0.0023$, p = 0.962) or Interaction effect ($F_{2, 18} = 0.699$, p = 0.510). A one-way ANOVA revealed a significant effect of Treatment in BACHD mice but not WT mice A one-way ANOVA revealed a significant Treatment effect in BACHD mice ($F_{4, 16} = 4.840$, p = 0.015) but no significant Treatment effect in WT mice ($F_{4, 16} = 1.249$, p = 0.342). Posthoc testing using the Bonferroni Multiple Comparison Test revealed that 0.3 mg/kg and 3.0 mg/kg 8-OOH-DPAT significantly increased the time spent in the open arm of EZM compared to vehicle-treated BACHD mice.

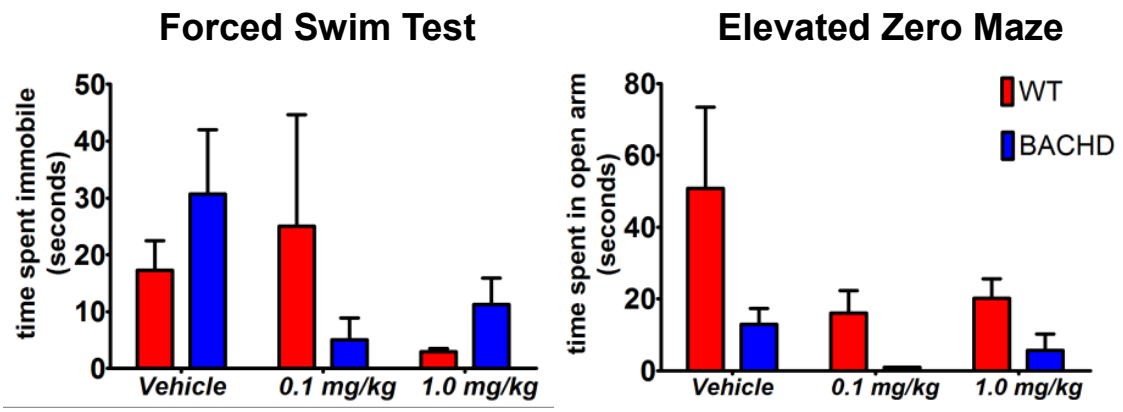

Figure 5.1 Effect of selective serotonin reuptake inhibitor on immobility in the FST and exploration in the EZM in WT and BACHD mice. Time spent immobile in the FST and Duration of time spent in the open arm of the EZM following administration of vehicle (saline), 0.1 mg/kg and 1 mg/kg escitalopram. There were no significant Treatment or Genotype effects on immobility in FST in WT or BACHD mice.

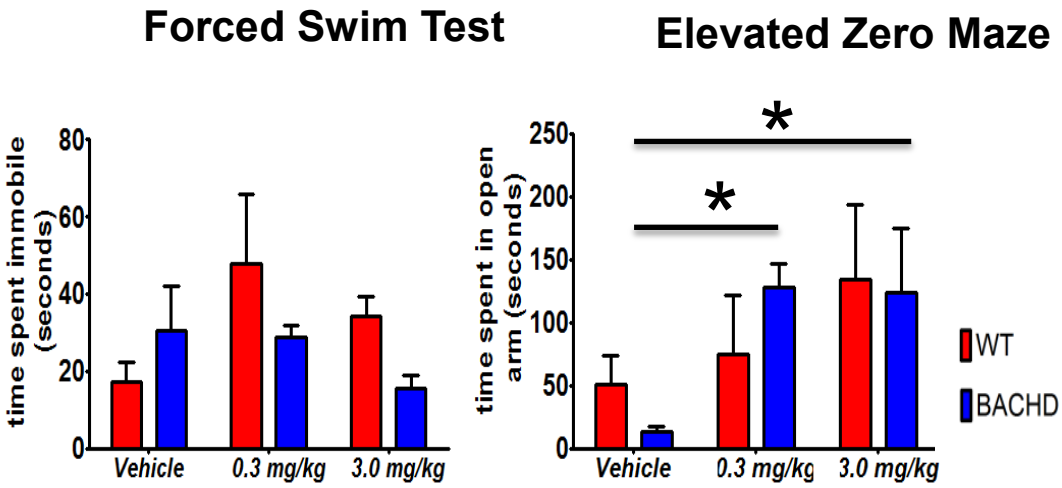

Figure 5.2 Effect of 5-HT1A agonist on immobility in the FST and exploration inthe EZM in WT and BACHD mice. Time spent immobile in the FST following administration of vehicle (saline), 0.3 mg/kg and 3.0 mg/kg 8-OH-DPAT. There was no significant effect of either treatment of 8-OH-DPAT on the time the WT and BACHD mice spent immobile in the FST. 0.3 mg/kg and 3.0 mg/kg 8-OH-DPAT significantly

increased the time the BACHD mice spent in the open arm of the EZM. * Indicates $p < 0.05$. * indicates within-group differences from duration in the open arm compared to time mice treated with vehicle spent in the open arm.

5.3.3 Serotonin efflux increases in ventral hippocampus following administration of escitalopram but not 8-OH-DPAT

To better understand the therapeutic efficacy of escitalopram and 8-OH-DPAT, I investigated the effect of both drugs on serotonin efflux. *In vivo* microdialysis was used to measure serotonin efflux in order to detect changes in serotonin neurotransmission. Escitalopram was administered via reverse dialysis through the microdialysis probe into the ventral hippocampus. This was done because the target of escitalopram, the serotonin transporter, is expressed on the cell terminals of raphe neurons that are located in the ventral hippocampus. 2-way ANOVA was used to compare the change in serotonin efflux in the WT and BACHD mice following administration of 0.1 μM, 1.0 μM and 10 μM escitalopram. For 0.1 μM, a 2-way ANOVA revealed a significant effect of Treatment (Figure 5.3, $F_{8,48} = 8.746$; $p < 0.0001$) but no significant effect of Genotype ($F_{1,48} = 0.107$, $p = 0.755$) or a significant Interaction effect ($F_{8,48} = 1.133$; $p = 0.359$). One-way ANOVA was used to analyze the effect of treatment on serotonin efflux separately in the WT and BACHD mice. In the WT mice, there was a significant effect of 0.1 μM escitalopram on serotonin efflux. Multi-comparisons posthoc testing using the Bonferroni alpha-correction method revealed that serotonin efflux significantly increased in the vHPC 45 minutes during drug infusion and that this effect persisted for 15 minutes in the post-infusion stage of pharmacological testing. In the BACHD mice, there was a

significant effect of 0.1 μM escitalopram on serotonin efflux 60 minutes following drug infusion, but this effect was not evident in other samples.

There was a dose-dependent effect of escitalopram treatment on serotonin efflux. For the dose of 1.0 μM escitalopram, 2-way ANOVA revealed significant effect of Treatment on serotonin efflux (Figure 5.3, $F_{8,72} = 20.48$; $p < 0.0001$) but no significant effect of Genotype ($F_{8,72} = 0.124$; $p = 0.732$) or a significant Interaction effect ($F_{8,72} = 1.545$; $p = 0.157$). One-way ANOVA of the effect of 1.0 μM escitalopram revealed a significant effect of Treatment in both WT ($F_{9,40} = 22.25$; $p < 0.0001$) and BACHD mice ($F_{8,32} = 6.949$; $p < 0.0001$). Posthoc testing using the Bonferroni alpha-correction method revealed that serotonin efflux was significantly increased in the WT mice 30 minutes during drug infusion and this persisted 45 minutes post drug infusion. In the BACHD mice, serotonin efflux was significantly increased 45 minutes following drug infusion of 1.0 μM escitalopram, which persisted 45 minutes post drug infusion. For the dose of 10.0 μM escitalopram, 2-way ANOVA revealed a significant Treatment effect (Figure 5.3; $F_{8,72} = 14.85$; $p < 0.0001$). There was no significant effect of Genotype ($F_{1,72} = 0.010$; $p = 0.762$) or a significant Interaction effect ($F_{8,72} = 0.550$; $p = 0.812$). One-way ANOVA was used to examine if this treatment effect was significant in either the WT or BACHD mice. There was a significant effect of 10.0 μM escitalopram in both WT ($F_{8,24} = 5.473$; $p = 0.0052$) and BACHD mice ($F_{8,24} = 4.5443$; $p = 0.0018$). Posthoc testing using the Bonferroni alpha-correction method revealed that serotonin efflux was significantly increased in the WT mice immediately during infusion and this effect persisted 60 minutes post infusion. Posthoc testing using the Bonferroni alpha-correction method revealed that serotonin efflux was significantly increased in the BACHD mice

immediately 30 minutes during infusion and this effect persisted 30 minutes post infusion.

The same analysis was used to observe the effect of 8-OH-DPAT on serotonin efflux in the ventral hippocampus in the WT and BACHD mice. For 0.3 mg/kg 8-OH-DPAT, 2 way ANOVA revealed that the drug treatment did not have a significant effect on serotonin efflux in either WT or BACHD mice (Genotype: $F_{1,18} = 3.417$, $p = 0.081$; Treatment: : $F_{8,18} = 0.852$, $p = 0.572$; Interaction: : $F_{1,18} = 0.818$, $p = 0.597$). For 3.0 mg/kg 8-OH-DPAT, 2 way ANOVA revealed a significant effect of Genotype on serotonin efflux ($F_{1,18} = 6.081$, $p = 0.024$) but no significant Treatment or Interaction effect (Treatment: $F_{8,18} = 0.739$, $p = 0.658$; Interaction effect ($F_{1,18} = 0.553$, $p = 0.801$).

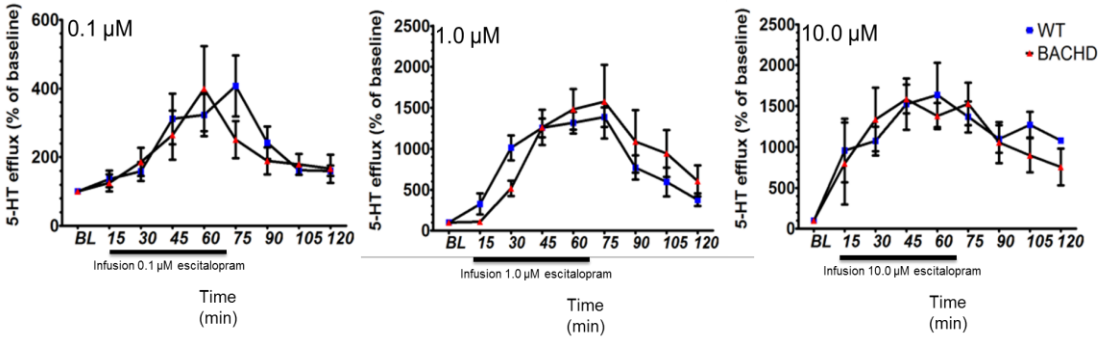

Figure 5.3 Effect of selective serotonin reuptake inhibitor, escitalopram on serotonin efflux in the vHPC in WT and BACHD mice. Change in serotonin efflux in the ventral hippocampus following the local administration of 0.1 µM, 1.0 µM and 10.0 µM escitalopram.

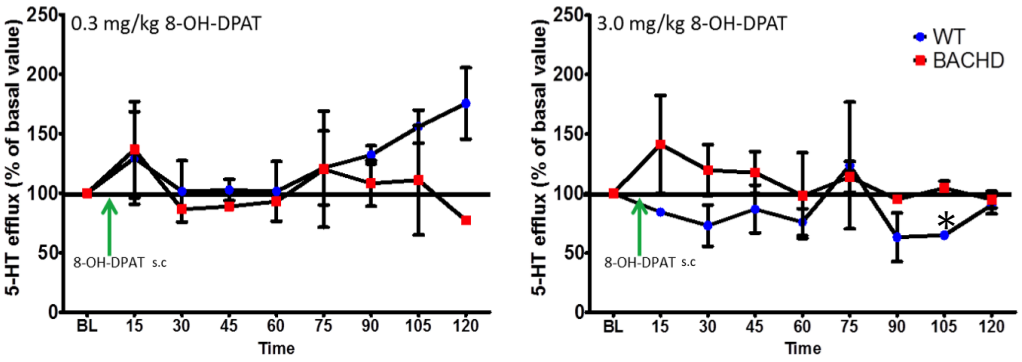

Figure 5.4 Effect of 5-HT1A agonist, 8-OH-DPAT, on serotonin efflux in the vHPC in WT and BACHD mice. Change in serotonin efflux following the systemic administration of 0.3 mg/kg and 3.0 mg/kg 8-OH-DPAT. * indicates within-group effect of treatment on serotonin efflux relative to baseline in WT mice ($p < 0.05$).

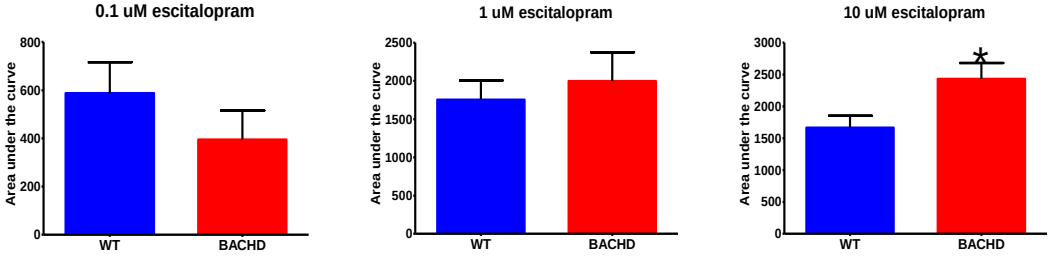

Figure 5.5 Area under the curve for treatment with 0.1 uM, 1.0 uM, and 10.0 uM escitalopram. The area under the curve following the local administration of 10.0 uM escitalopram significantly differed between WT and BACHD mice with higher area under the curve value for the BACHD mice (*: $p < 0.05$).

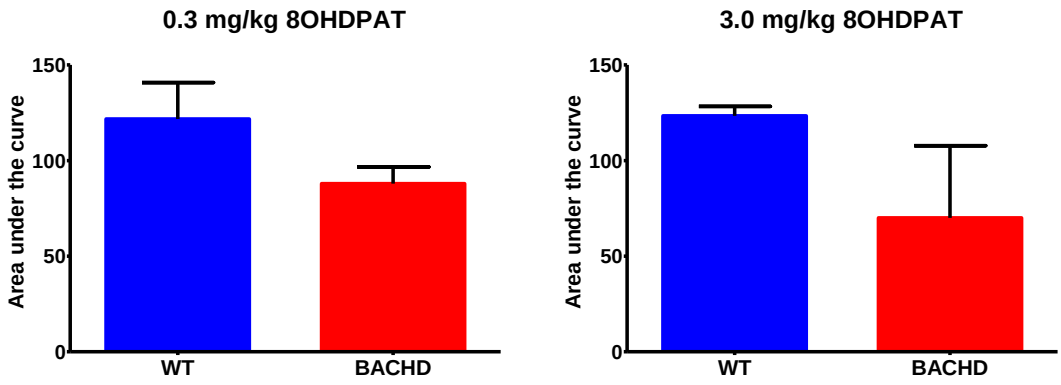

Figure 5.6 Area under the curve for treatment with 0.3 mg/kg and 3.0 mg/kg 8-OH-DPAT. The area under the curve values did not significantly differ between WT or BACHD mice following treatment with 8-OH-DPAT at either dose.

The area under the curve value (AUC) was significantly higher in the BACHD mice as a result of treatment with 10.0 uM escitalopram [$t(6) = 2.67$, $p = 0.049$] but not 0.1 [$t(9) = 0.56$, $p = 0.59$] or 1.0 uM [$t(6) = 1.09$, $p = 0.32$] escitalopram (Figure 5.5). AUC values with treatment of 0.3 mg/kg [$t(2) = 1.62$, $p = 0.25$] and 3.0 mg/kg [$t(2) = 1.4$, $p = 0.29$] 8-OH-DPAT did not significantly differ between WT and BACHD mice (Figure 5.6).

5.4 Summary of Results

The therapeutic efficacy of the SSRI, escitalopram and the 5-HT1A receptor agonist to alleviate behavioral abnormalities in the BACHD translational HD mouse model was explored using behavioral and neurochemical tests. The anxiolytic property of both drugs was determined by measuring the duration the mice spent in the open arm of the elevated zero maze following treatment with varying doses of escitalopram and 8-OH-DPAT. 0.3 mg/kg 8-OH-DPAT and 3.0 mg/kg 8-OH-DPAT significantly increased the time the BACHD mice but not the WT mice spent in the open arm of the elevated

zero maze. The various doses of escitalopram did not have an anxiolytic effect in either the WT or BACHD mice. The antidepressant properties of 8-OH-DPAT and escitalopram were assessed by measuring the effect of both treatments on time spent immobile relative to vehicle-treated WT and BACHD mice. Neither treatment did not significantly reduced the time spent immobile in the WT and BACHD mice.

Interestingly, there is evidence of a non-significant trend that 3.0 mg/kg 8-OH-DPAT. 0.1 mg/kg and 1.0 mg/kg escitalopram reduced the time spent immobile in the BACHD mice compared to vehicle-treated BACHD mice, and BACHD mice treated with 0.3 mg/kg 8-OH-DPAT. There is also evidence that 0.3 mg/kg, 3.0 mg/kg 8-OH-DPAT and 1 mg/kg escitalopram reduced the time spent immobile in the BACHD mice compared to WT mice treated with the same doses of 8-OH-DPAT and escitalopram. Only 1 mg/kg escitalopram had a larger effect on depressive-like phenotype in the WT mice than the BACHD mice. Conversely, there is evidence of a non-significant trend that both doses of 8-OH-DPAT increased the time WT mice spent immobile in the FST. The few number of mice tested in each treatment could account for the lack of significant effects. However, this suggests the efficacy of 8-OH-DPAT as an anxiolytic and antidepressant in the BACHD mice, whereas escitalopram only exhibited antidepressant properties in the BACHD mice.

Dysfunction in serotonin signaling is thought to underlie depression and anxiety. Several drugs that alleviate these symptoms function by increasing serotonin neurotransmission in various brain regions. One such region is the ventral hippocampus, which is functionally connected to the prefrontal cortex, amygdala and raphe, regions that

each has a role in the regulation of affect and mood. It is hypothesized that dysfunction in serotonin signaling in regions such as the ventral hippocampus contributes to the manifestation of affective disorders (Kirby and Lucki 1995; Amat et al. 1998; Kagamiishi et al. 2003). Therefore, I measured serotonin efflux, an analog of serotonin neurotransmission, in the ventral hippocampus in baseline conditions and following the administration of escitalopram and 8-OH-DPAT.

Though there were no substantial changes in serotonin efflux in either the WT or BACHD mice, there were some differences in the neurochemical response to 8-OH-DPAT between the BACHD and WT groups. The neurochemical response to 0.3 mg/kg 8-OH-DPAT was similar in both the WT and BACHD mice. For two hours post-injection, 3.0 mg/kg 8-OH-DPAT decreased serotonin efflux in the WT mice and increased serotonin efflux in the BACHD mice.

0.1 μM escitalopram moderately increased serotonin efflux in the vHPC of the WT and BACHD mice. Serotonin efflux was measured for 60 minutes during the active diffusion of escitalopram into the vHPC in WT and BACHD mice. This effect was modest in the WT and BACHD mice as the effect only significantly increased serotonin efflux for 45-60 minutes following the initiation of drug infusion. This time point is when the peak effect of 0.1 μM escitalopram on serotonin efflux was reached. In the BACHD mice, 0.1 μM escitalopram only significantly increased serotonin efflux for one time point, 60 minutes post infusion. Interestingly, the peak effect of this dose was 60 minutes after infusion in the BACHD mice and 15 minutes post infusion in the WT mice, when escitalopram was washed out of the vHPC. The effect of escitalopram on serotonin efflux

in the ventral hippocampus was dose-dependent as 1.0 μM and 10.0 μM escitalopram elicited a larger increase in serotonin efflux. However, the effect of 1.0 μM and 10.0 μM escitalopram was similar. In the WT and BACHD mice, 1.0 μM and 10.0 μM escitalopram elicited a ~ 1500% increase in serotonin efflux in the vHPC. This percent change was did not significantly differ between the WT and BACHD mice. Additionally, the peak increase in serotonin efflux from 1.0 μM escitalopram in both WT and BACHD mice was 15 minutes after escitalopram was washed out. The peak increase in serotonin efflux from 10.0 μM escitalopram in the WT and BACHD mice was 60 minutes following the infusion of escitalopram. AUC values revealed a slightly larger increase in the BACHD mice following treatment with 10.0 uM escitalopram, suggesting a larger response to escitalopram in the BACHD mice at the highest dose.

5.5 Commentary

One shortcoming of the behavioral and neurochemical experiments detailed in this chapter was the low number of WT and BACHD mice used. The recommended number of mice per experimental group (per genotype for each drug treatment and dose) is 12-15 mice to ensure significant power for an effect of treatment to be detected (Suzoff Rizzo and Crawley 2017). In these experiments 2-4 mice were used per experimental group (vehicle and both doses of 8-OH-DPAT and escitalopram). This could account for the lack of significant effect of drug treatment on the depressive-like phenotype in the BACHD mice. Interestingly, despite the low subject number, the anxiolytic properties of 8-OH-DPAT were evident in the BACHD mice.

Further evidence of the involvement of the 5-HT1A receptor in the etiology of affective disorder will require additional experiments using agonists and antagonists of the 1A receptor. In interest of being thorough, it should have been tested if 5-HT1A antagonists augment the anxious and depressive phenotype of the BACHD mice. This would provide additional support in the role of the serotonin, acting via the 5-HT1A receptor, in the regulation of mood and affect in the BACHD mice. It is also possible that 8-OH-DPAT did not have a significant antidepressant because the target of this drug, the 5-HT1A, is functionally altered in the BACHD mice Postsynaptic 5-HT1A receptor expression is decreased in other HD mouse models (Pang et al. 2009; Renoir et al. 2010; Renoir et al. 2013). This can be tested using drugs that would mimic the effect of serotonin or 8-OH-DPAT binding at the postsynaptic receptors. However, this requires detailed knowledge of the circuitry involved in this behavior. The 5-HT1A receptor is expressed on glutamatergic pyramidal neurons GABAergic local interneurons in the hippocampus (Berumen et al. 2012). However, there is only speculation if depression involves those two neuronal populations. Serotonin inhibits neuronal firing of neurons that express the 5-HT1A receptor as the metabotropic receptor is that is coupled to the inhibitory G_i/G_o protein subunit, which inhibits cyclic adenosine monophosphate (cAMP) activity (Hoyer 2010). It would still be useful to locally inhibit neurons in the vHPC and see what effect it would have on activity mobility in the FST.

CHAPTER VII:

General Discussion

The primary objective of this dissertation was to investigate in detail the behavioral and neurochemical profile of the BACHD mouse model as it pertains to the validity of this translational mouse model. The validation of this mouse model would support the use of this as a tool for early preclinical studies on the pathophysiology of affective disorders in HD. Importantly these studies were informed by preclinical and clinical studies of affective symptoms in HD patients and translational mouse models. This study was also largely informed by the numerous preclinical and clinical studies on idiopathic psychiatric symptoms that develop in absence of neurodegenerative diseases. From the aforementioned studies, several potential targets are implicated in the etiology of affective disorders that can be targeted for therapeutic intervention. Another use of a valid translational mouse model was to assess the efficacy of existing treatments and to identify novel treatments for these symptoms. This was a secondary goal of this dissertation.

6.1 Potential neuropathological changes in the brain of HD patients that exhibit affective symptoms

The development of the psychiatric symptoms in HD is contingent upon the presence of the genetic mutation and is likely not influenced by the stressful circumstances of inheriting a fatal disease (Marshall et al. 2007; Julien et al. 2007; van Duijn et al. 2008). This was determined by clinical studies in which patients develop the psychiatric symptoms in absence of knowledge of their disease status. This suggests that these symptoms are purely pathologically driven. Given the psychological nature of these symptoms, it is likely that the symptoms manifest due to these changes occurring in

limbic-related structures in HD patients. The genetic mutation alters the morphology of the ubiquitous huntingtin protein (HTT) in the brain (Sandou and Humbert 2016). The most common way the mutation affects neuronal function is through the formation of intracellular protein aggregates, which facilitates the activation of autophagy pathways to result in cell death (Pla et al. 2014). There is the potential that the mutant protein alters neuronal function in limbic structures, similar to how mHTT protein expression drives the loss of MSNs in the caudate and putamen in patients (Vonsattel 2008).

The primary neuromodulators in limbic-related circuits in the brain includes serotonin and norepinephrine. Reduced serotonergic activity in the brain is largely implicated in abnormal limbic function and the incidence of affective disorders in otherwise healthy patients (Hamon and Blier 2013). It is largely unknown if serotonin is lost in the brain of HD patients as this has only been investigated in a few studies (Krogais et al. 2011). In this experiment, there is evidence that serotonergic activity is reduced in the BACHD mouse model with comorbid anxiety and depression. However, subsequent experiments failed to find a significant association with serotonin levels in the limbic system and the display of anxious and depressive behaviors in the HD mice. Further investigation was undertaken to better understand the role of this impairment in serotonergic function in the HD mice.

6.2 BACHD mouse model represents a valid translational mouse model of affective disorder in HD

The initial step of this study was to experimentally validate the use of the BACHD mice for the investigation of the psychiatric symptoms of HD. The criteria for

validating the use of a translational mouse model include 1) face, 2) construct and 3) predictive validity. Each chapter in this dissertation addressed one criterion for the use of the BACHD mouse model in the investigation of the etiology of psychiatric symptoms in HD. With the data presented, it appears that the BACHD mouse model meets the criteria for face, construct and predictive validity.

6.2.1 BACHD mouse model mimics the development of motor and psychiatric symptoms in HD patients

The first criterion for assessing the validity of a translational mouse model is face validity. In this study, that means the HD mice must show an equivalent behavioral phenotype that closely mimics the mood abnormalities experienced by patients. Approximately 3%-11% of HD patients experience aggression, irritability, apathy, and the two most common symptoms; anxiety and depression. This occurs 5-15 years before the onset of motor chorea in conjunction with other minor motor difficulties (Cranfurd et al. 2001; Kulisevsky et al. 2001: Murgod et al. 2001; Paulsen et al. 2001). I conducted these studies using the BACHD mouse model because it was previously shown that this model exhibits motor, psychiatric and cognitive symptoms (Gray et al. 2008). However, only a few studies investigated the psychiatric and motor abnormalities in this mouse model in the same study.

The BACHD mice were concurrently tested in the fixed-speed rotarod task, the elevated zero maze, and the forced swim test at the same age, 8 weeks old. It was evident that at this age, the BACHD mice exhibit psychiatric-like symptoms in the presence of minor motor deficits. I also monitored the motor performance of the BACHD mice

longitudinally using the fixed-speed rotarod task. The motor symptoms that are present at 8 weeks are moderate compared to later ages (12-24 weeks old). The presence of progressive motor deficits with simultaneous psychiatric-like symptoms supports that the BACHD mice exhibit an endophenotype that is comparable to the development of the motor and psychiatric symptoms in HD patients. Importantly, the psychiatric symptoms manifest in the prodromal stage of HD (prior to the onset of chorea which marks the manifestation of the motor symptoms). Therefore, it is possible that the BACHD mice develop symptoms of the equivalent prodromal stage at 8 weeks old. However, this is difficult to assess, as it requires identifying the ages in the mice and humans that are developmentally similar.

The average age for chorea (clinical onset of HD) in HD patients is 45-50 years old (Julien et al. 2007; Tabrizi et al. 2011; Thompson et al. 2012; Dale et al. 2016). 30-72 weeks old in mice is considered developmentally similar to middle age in humans (Sukoff Rizzo and Crawley 2017). The ages at which the psychiatric symptoms typically manifest occurs in the early 30s or 40s, 5-10 years before the motor symptoms. In mice, the approximate equivalent age to the third and fourth decade of life in humans is 12-30 weeks old. Theoretically, this is when it can be expected for psychiatric symptoms to manifest in the mice. However, I observed this behavioral phenotype in the BACHD mice earlier at 8 weeks old. If 12-30 weeks old in the mice is considered to be the equivalent age for the prodromal stage in HD patients, then my findings provide evidence that the mice may develop the symptoms sooner. There is evidence that the BACHD mice may develop anxious and depressive symptoms as early as 4 weeks old (Menalled et al. 2009). Future experiments should be conducted to compare the 8-week age point in

the mice to earlier and later ages to accurately pinpoint when these symptoms first manifest. The most effective method to determine the ideal age would require a longitudinal study of the anxious-like and depressive-like symptoms in the BACHD mice.

It is not entirely unexpected for the BACHD mice to develop symptom in a rapid progression given the drastically shorter lifespan of mice but also because most of the HD transgenic mouse models have a higher CAG repeat length than observed in humans. In humans, HD manifests with a CAG repeat length of approximately 40 (Vonsattel 1985). The BACHD mouse model has 97 CAA/CAG repeats (Gray et al. 2008). There is a direct positive correlation between the CAG repeat length and the age of onset with a longer repeat length associated with an earlier onset. As true as this is in HD patients, it is equally true in the HD mouse models. The R6/2 HD mouse model exhibits the most rapid development of HD with onset of motor deficits prior to 4 weeks old and death early at 11-12 weeks old (Mangiarini et al. 1996). This mouse model is considered to represent the rare form of juvenile HD, in which symptoms emerge in early childhood in individuals with more than 70 CAG repeats (Sturrock and Leavitt 2010).

The average age of the clinical studies in prodromal HD patients was rarely younger than 34 (Paulsen et al. 2008; Duff et al. 2007; Tabrizi et al. 2011; Scahill et al. 2013; Epping et al. 2016; Martinez-Horta et al. 2016). Therefore, it has not been investigated if HD patients show symptoms outside the expected 5-15 year period before chorea. There is a benefit to knowing when symptoms first emerge, as this would be necessary in order to determine when therapeutic measures are needed. Perhaps

additional clinical studies should be conducted to determine if HD patients show mood abnormalities earlier in adulthood.

To summarize, our studies identify that 8 weeks old was a useful age for the purpose of this study because the BACHD mice were not severely impaired in motor ability and did show evidence of behavioral deficits in anxiety and depressive tests. However, given the results from other studies, it cannot be determined if this age point is the earliest that the psychiatric-like symptoms manifest and the most closely related age of the prodromal stage.

6.2.2 Investigation of the etiology of psychiatric symptoms of HD using the BACHD transgenic mouse model

The construct validity of the pathophysiology of the psychiatric symptoms in HD is largely unknown due to the dearth of information available from clinical studies in HD patients. Conversely, there is a breadth of information available on the neurobiology of idiopathic affective disorders. That information, in conjunction with the information available from other studies in the transgenic HD mouse models, was used to identify potential neurobiological underpinnings of the disease. Most of those studies in both clinical and preclinical animal studies investigated deficits in serotonergic neurotransmission in relation to affective disorders.

Therefore, the experiments undertaken here were designed to investigate if serotonergic signaling is impaired in the symptomatic BACHD mice. This was achieved using *in vivo* microdialysis to measure serotonin efflux in the ventral hippocampus in

wildtype and HD mice. Indeed, in the symptomatic BACHD mice, there is evidence of impaired serotonergic signaling in the ventral hippocampus. It is hypothesized that the DRN and MRN regulate affect through serotonergic projections to several regions including the ventral hippocampus (Lowry and Hale 2010).

Serotonergic signaling can be disrupted in affective disorders through several mechanisms, including altered reuptake via the serotonin transporter, impaired neuronal firing, altered metabolism due to dysfunction of the monoamine oxidase enzyme, and dysfunction at any of the 14 serotonin receptor subtypes in the brain (Lesch et al. 2003). Of those features, impaired status of the 5-HT1A receptor (Cross et al. 1986; Waeber and Palacios 1989) and increased metabolism of serotonin by monoamine oxidase are implicated in HD patients (Richards et al. 2011). In the HD mouse models, serotonin was implicated due to evidence of reduced serotonin concentration in the cortex and hippocampus in the R6/1 and R6/2 mouse model (Pang et al. 2009; Renoir et al. 2011; Pouladi et al. 2012) and my finding that serotonin efflux in reduced in the ventral subdivision of the hippocampus. Importantly, this indicates that serotonin activity can be disrupted by means of several mechanisms to manifest in anxiety and depression in HD and idiopathic anxiety disorders. This is an important distinction that implicates serotonin in affective disorders and reveals the complexity of the involvement of serotonin in affect that is not explained by the monoamine hypothesis of affective disorders. The deficit in serotonergic neurotransmission in the vHPC of the BACHD mice is evidence that this mouse model does meet the criterion for construct validity. A deficit in serotonin activity in the vHPC implicates multiple mechanisms of serotonergic neurotransmission including

dysfunction of the 5-HT1A receptor, impaired reuptake by SERT, and reduced serotonin release.

6.2.3 BACHD mouse model meets criterion for predictive validity

Most HD patients that experience depression and anxiety are medicated with selective serotonin reuptake inhibitors (Rowe et al. 2012). To test the predictive validity of the BACHD mice mouse model, it was necessary to examine if pharmacological treatments had a similar effect in the HD mice. Acute administration of the SSRI escitalopram non-significantly decreased immobility and decreased exploration in the EZM in WT and BACHD mice. This is indicative of a potential antidepressant effect and anxiogenic response in both groups. This effect was likely not significant due to the low number of mice tested in each treatment. Testing with more mice will likely yield a significant effect given the degree of change in immobility and exploration following treatment with the agonist 8-OH-DPAT in the 2-4 mice tested in each group. SSRI treatment also successfully attenuated the depressive but not the anxious behaviors in the BACHD model and other mouse HD models. The acute and chronic administration of the SSRI sertraline alleviated depressive-like symptoms in female and male R6/1 mice in the FST and TST (Pang et al. 2009; Renoir et al. 2010). Acute treatment with the SSRI escitalopram augmented anxious behaviors of the BACHD mice in the EZM. Therefore, this HD mouse model and other mouse models do show a similar antidepressant response to SSRI treatment. However, HD patients also show an anxiolytic response to SSRIs that was not evident in this study. The anxiolytic effect of SSRI treatment is evident with chronic SSRI administration. The effect of chronic SSRI treatment on the anxiogenic

phenotype of HD mice has not been examined. Therefore, whilst the BACHD mouse model and other mouse models show a similar behavioral response to SSRI treatment in humans, it remains to be investigated if they respond the same to anxiolytics. Therefore, the BACHD mouse can be used to assess the therapeutic efficacy of SSRI treatment and can likely be used to investigate other therapeutic interventions. The similar response of SSRI treatment in HD patients and BACHD mice suggests a similar pathological mechanism underlying the psychiatric symptoms of HD. Simplistically, the efficacy of SSRI treatment in both HD patients and HD mice supports evidence of an underlying deficit in serotonergic neurotransmission. Additional studies were needed to elucidate what aspects of serotonergic neurotransmission are impaired.

6.3 Serotonergic dysfunction in limbic structures in the BACHD mice is not directly related to the incidence of affective disorder in HD

The evidence that the BACHD mice are behaviorally similar to humans in the early prodromal phase of HD warrants the use of this mouse model to investigate the neurobiology of these symptoms. In humans, imaging studies in living HD patients and postmortem morphological studies has yielded little about the biological underpinnings of the psychiatric symptoms. Those studies failed to identify a correlation between the morphological and functional changes in cortical and basal ganglia structures that were implicated in the cognitive and motor symptoms (Paulsen et al. 2008; Rosas et al. 2005; Beglinger et al. 2013; Tang and Feigin 2012). The morphological changes that are significantly associated with the psychiatric symptoms include alterations in raphe activity and in serotonergic activity as evident by reduced echogenicity in the raphe and

reduced serotonin levels in patients (Kurlan et al. 1988; Garrett and Soares-da-Silva 1992; Garcia Ruiz et al. 1995; Krogias et al. 2011). This implicates impaired serotonergic activity in the etiology of the psychiatric symptoms of HD. However, from my findings, it was evident that serotonin levels and the incidence and severity of the anxious and behaviors in the mice are not directly related. Yet, there are relevant consequences of reduced serotonin efflux that can be discussed in the incidence of affective disorders.

6.3.1 Mechanisms of impaired serotonergic neurotransmission in HD

From the microdialysis experiments, it was evident that different mechanisms of serotonergic neurotransmission are impaired. Serotonin efflux in the BACHD mice was decreased by ~30% compared to the wildtype mice (Figure 4.2). The amount of the serotonin metabolite 5HIAA in the BACHD mice was comparable to 5HIAA levels in the wildtypes (Figure 4.2). This suggests that serotonin is metabolized at a higher rate in the BACHD mice than wildtypes. Impaired metabolism of serotonin was previously implicated in affective disorders in HD patients and healthy individuals (Du et al. 2013). 5-HIAA levels were reduced in CSF of HD patients, similar to depressed individuals (Asberg et al. 1976; ; Caraceni et al. 1977; Jongen et al. 1980; van Praag et al. 1984; Roy et al. 1989). Furthermore, Richards and colleagues (2011) observed that monoamine oxidase (MAO) expression is elevated in HD patients, similar to healthy individuals (Meyer et al. 2006). Additionally, Vinther-Jensen and colleagues (2016) found a correlation between the psychiatric symptoms in HD patients and polymorphisms in the

gene for monoamine oxidase A (the primary subtype that metabolizes serotonin in the brain) that results in increased enzymatic activity.

It has been shown that MAO expression is increased in cultured striatal cells that express the mutant huntingtin protein and in HD patient-derived induced pluripotent stem cell lines (Ooi et al. 2015). It was also discovered that chronic treatment with the monoamine oxidase-subtype A inhibitor, clorgyline, reduced the incidence of depressive-like and anxiety-like symptoms as well as restored serotonin levels in the striatum of YAC128 mice (transgenic mouse model of HD) (Garcia-Mirelles et al. 2016). Also, in a case of three HD patients, the monoamine oxidase inhibitors (MAOIs) phenelzine and isocarboxizid alleviated symptoms of anxiety and depression within 7-9 days (Ford 1986). This therapeutic effect developed much quicker than the typical time it takes SSRIs to be efficacious. Therefore, one potential mechanism of impaired serotonin activity in HD appears to result from increased metabolism of serotonin, which can be successfully treated with MAOIs. MAOIs are a known class of antidepressants and anxiolytics in individuals with idiopathic psychiatric symptoms (Cryan and Holmes 2005).

The other mechanism of neurotransmission that may account for the reduced serotonin efflux in the BACHD mice is increased reuptake of serotonin by the serotonin transporter (SERT). Increased serotonin reuptake is implicated because SSRIs attenuate the incidence of affective disorders in HD patients and in idiopathic anxiety and depression (Rowe et al. 2012). However, pathological changes in SERT function are only implicated in individuals with psychiatric disorders that have certain genetic variants of

the SERT gene. Patients with Major Depressive Disorder that inherit the ‘s’ allele of the SERT gene have a higher incidence of depression and other affective disorders than carriers of other genetic variants (Young et al. 2008). The ‘s’ allele is associated with reduced levels of SERT expression (Dorado et al. 2007). While SERT dysfunction can be attributed to the incidence of psychiatric symptoms, it remains unknown if SERT is affected in HD (Pla et al. 2014) Clinical studies are needed to address if genetic variants of the serotonin transporter are present or if there is evidence of altered function of SERT in HD patients. As a result, increased reuptake is not suspected in the reduced serotonin efflux in the BACHD mice.

In HD patients, there is evidence of bidirectional alterations in serotonin activity with increased serotonin levels in the striatum and decreased serotonin in the hippocampus (Reynolds and Pearson 1987). To investigate if serotonergic activity is similarly affected in the BACHD mice, I measured extracellular serotonin in the dorsal striatum. There was no observed deficit in serotonin efflux in the BACHD mice in the dorsal striatum. This bidirectional effect on serotonin activity in patients may be related to the involvement of the different subdivisions of raphe nuclei in affective disorders. The dorsal striatum receives raphe projections from the dorsal, ventral, and caudal subdivision of the dorsal raphe (Muzerelle et al. 2014), whereas the vHPC receives afferents from the caudal and ventral subdivision of dorsal raphe, as well as the majority of its afferents from the median raphe (McKenna and Vertes 2001; Fanselow and Dong 2010). The primary difference in the innervation of these two structures is that the median raphe does not project to the striatum.

It is hypothesized that the median and dorsal raphe are involved in different aspects of affective behavior in rodent models. Paul and Lowry (2013) reviewed evidence of the involvement of distinct raphe subdivisions and serotonergic circuits in mood and affect. The caudal and dorsal subdivisions of DRN project to forebrain structures that are involved in anxiety-related behavior and so are hypothesized to have a role in the control of anxious behaviors (Paul and Lowry 2013). The MRN projects to forebrain structures involved in the behavioral response to stressful stimuli, which is implicated in depressive behavior (Paul and Lowry 2013). This may implicate functional alterations in the MRN and not the DRN in the behavioral phenotype in the BACHD mice. However, the vHPC also receives input from the caudal and ventral subdivisions of DRN, which suggests that the DRN also contributed to the observed phenotype in the BACHD mice. Therefore, it can only be speculated if serotonin efflux is affected in different target structures due to the origin of the serotonergic input as is suggested in this study. Additional experiments are needed to examine neuroanatomical and neurochemical changes in the different subdivisions of raphe in HD.

Additionally, I measured dopamine and the dopamine metabolite, DOPAC in the dorsal striatum. In the striatum, dopamine is involved in the modulation of the direct and indirect pathway of the basal ganglia (Albin et al. 1999). Given that the BACHD mice showed minor impairment in rotarod performance, it was suspected that dopamine function might be impaired at this age. However, there were no significant differences in dopamine efflux between the wildtypes and BACHD mice. It is not uncommon that patients show minor motor difficulties in absence of robust deficits in the caudate and putamen as most motor impairments do not manifest until approximately 40% of MSNs

are lost (Vonsattel 2008) much later than the early prodromal stage of HD. The rotarod deficits in the BACHD mice could be a function of their weight, as on average the BACHD mice weigh 20 - 30% more than wildtypes starting from 8 weeks old (Gray et al. 2008). The BACHD mice in this study did weigh approximately 20 - 40% more than the wildtypes at the time of rotarod testing.

Another mechanism of serotonin neurotransmission that may be affected in HD includes impaired serotonin release and impaired receptor binding. The former implicates a deficit in presynaptic serotonin activity whereas the latter implicates a deficit in postsynaptic serotonin activity. As discussed earlier, the contribution of serotonin depletion to anxiety and depression is still under speculation. For that reason, I chose to focus on the latter involvement of serotonin receptor binding in neurotransmission and in the involvement of the incidence of anxiety and depression in the BACHD mice.

6.3.2 Role of the 5-HT1A receptor in the BACHD mice

Serotonergic neuronal activity is highly regulated by 5-HT1A receptors, which acts as an inhibitory autoreceptor to reduce serotonin release via negative feedback mechanisms (Knobelman et al. 2001). 5-HT1A receptors are also inhibitory heteroreceptors in postsynaptic targets such as the vHPC (Hoyer et al. 2010). It appears that dysfunction of the 5-HT1A receptor is a causal link between comorbid anxiety and depression (Stahl 1997). Humans with a C-1019G single nucleotide polymorphism in the HTR1A gene (HTR1A-1019) exhibit severe depression and anxiety (Stroebel et al. 2003; Lemonde et al. 2003). This suggests that dysfunction of 5-HT1A receptors may underlie the etiology of the comorbid phenotype in the BACHD mice.

To investigate this, I examined if activation of the 5-HT1A, using the agonist 8-OH-DPAT, was sufficient to alleviate the anxiety-like and depressive-like behaviors of the BACHD mice. 8-OH-DPAT elicited an anxiolytic effect in the BACHD mice but had no significant antidepressant effect. From the literature, it is suspected that the anxiolytic property of 8-OH-DPAT is facilitated by the 5-HT1A autoreceptor by inhibiting serotonin release (Sharp et al. 1989). However, there is the possibility that 8-OH-DPAT alleviated the anxious behaviors of the BACHD mice by means of the heteroreceptor. Local infusion of 8-OH-DPAT into the hippocampus (activation of the 1A heteroreceptor) significantly increased rodent exploration in the EPM (Menard and Treit 1998). Additional experiments can be conducted to determine if the activation of the heteroreceptor has an anxiolytic and antidepressant effect. Specifically, the effect of 5-HT1A receptor activation on the affective phenotype in the BACHD mice should be conducted by locally infusing the drug in the vHPC in lieu of systemic administration. If this manipulation has a similar anxiogenic effect and a significant antidepressant effect, this could implicate that impaired communication of the 5-HT1A receptor in postsynaptic targets contributes to the incidence of anxiety and depression in the BACHD mice.

The heterogeneous role of the 5-HT1A receptor offers interesting insight into how serotonin regulates affect and what changes in serotonergic signaling may facilitate the manifestation of psychiatric symptoms such as anxiety and depression. Specifically, the anxiolytic effect of the activation of the 5-HT1A heteroreceptor suggests that serotonin inhibits anxiety-related behavior through activation of this receptor on postsynaptic targets. This is further supported by other studies, in which downregulation of the 5-HT1A heteroreceptor in hippocampus is associated with anxiety-related behavior (Fuss et

al. 2013), whereas the overexpression of the receptor attenuates anxiety (Kusserow et al. 2004).

6.4 Affective symptoms in the BACHD mice can be treated with serotonergic and non-serotonergic therapeutic interventions

This role of the 5-HT1A heteroreceptor implicates the involvement of other neurotransmitter systems such as glutamate and GABA. The postsynaptic heteroreceptor in the vHPC is expressed on glutamatergic pyramidal neurons and GABAergic interneurons (Berumen et al. 2012). In the BACHD mice, which exhibit anxious and depressive behaviors, activation of 5-HT1A receptors globally elicited an anxiolytic and had a potential antidepressant effect. I have provided evidence that this is due, in part, to activation of the postsynaptic receptor. This implicates the involvement of both the GABAergic system and the glutamatergic system in affective disorders in HD.

6.4.1 Role of serotonin, glutamate and GABA in the ventral hippocampus

The similarity in the neuronal composition of the vHPC and PFC is indicative of the analogous role of these two structures to regulate mood and affect. The principle neurons in the vHPC and PFC are glutamatergic projection neurons and GABAergic interneurons (Weisstaub at al. 2006). In the vHPC and PFC, primarily the medial PFC (mPFC), these neurons express the 5-HT1A, 5-HT1B, and 5-HT2A heteroreceptors (Amargos-Bosch et al. 2004; Santana et al. 2004) in addition to other receptor subtypes of the 5-HT1 and 5-HT2 family (Weisstaub et al. 2006; Berumen et al. 2012). However,

given that I only tested the role of the 5-HT1A receptor in HD, I limited my discussion to that receptor.

Albert et al. (2014) proposed a model of anxiety and depression in which reduced serotonin availability in the mPFC drives the manifestation of affective symptoms due to the differential inhibition of the glutamatergic and GABAergic neurons. In instances of low serotonin availability, serotonin would selectively inhibit GABAergic interneurons, which are hypothesized to have a greater affinity for serotonin than glutamatergic pyramidal neurons. This inhibition would in turn disinhibit the glutamatergic neurons, leading to overactivity in anxiety-related circuits. In depression-related circuits, low serotonin availability would result in reduced inhibition of GABAergic interneurons (not enough serotonin to bind). This would drive the increased inhibition of the pyramidal neurons, which is thought to have a pro-depressant effect.

The model proposed by Albert et al. (2014) could explain why acute administration of the 5-HT1A agonist, 8-OH-DPAT, was anxiolytic and antidepressant in the BACHD mouse model. 8-OH-DPAT is shown to act primarily through postsynaptic 5-HT1A receptors to elicit am antidepressant effect (Luscombe et al. 1998). Similar the proposed model, the BACHD mouse model has reduced serotonin efflux in the vHPC. For the purpose of this explanation, I am speculating that the behavioral affect on serotonergic regulation in the vHPC is comparable to the proposed model of low serotonin activity in the mPFC. If reduced serotonin neurotransmission drives the manifestation of anxious and depressive behaviors due to failure of serotonin to regulate the inhibition and disinhibition of pyramidal neurons in vHPC, utilizing a 5-HT1A

agonist could theoretically restore this communication and alleviate the behavioral symptoms. This can be tested by locally administering the 5-HT1A agonist, 8-OH-DPAT, in the vHPC of the BACHD mice and observe the effect this has on the anxious and depressive phenotype of the HD mice.

6.5 Implications for the treatment of psychiatric symptoms in HD

To summarize, the experiments detailed in this dissertation provide evidence that anxiety and depression are related, but not directly driven by impaired serotonergic neurotransmission in postsynaptic targets such as the ventral hippocampus. The primary cell types in the ventral hippocampus are glutamatergic projection neurons and GABAergic interneurons that receive dense innervation from serotonergic neurons and communicate via the 5-HT1A receptor. Taken together, this implicates that altered communication between raphe and the ventral hippocampus, which comprise behaviorally-relevant limbic circuits, may contribute to the incidence of affective disorders such as anxiety and depression in HD. Novel treatments that restore communication between those regions may function by targeting the serotonin receptors that are expressed in those regions. The main receptor subtype that is expressed on both neuronal types in the ventral hippocampus is the 5-HT1A receptor subtype (Berumen et al. 2012). Serotonin is thought to modulate activity in the ventral hippocampus by regulating inhibition between the two groups to maintain balanced communication. Dysfunction of that communication by reduced serotonin efflux may be the underlying etiology of these symptoms in HD. This is supported by the potential antidepressant and anxiolytic response of 8-OH-DPAT in the BACHD mice.

6.6 Implications for idiopathic affective disorders

The studies that others and I have conducted on the psychiatric symptoms of HD using the mouse models may lead to discoveries that can consequently inform the etiology of idiopathic affective disorders. The BACHD mouse model and the other HD transgenic mouse models are unique in the fact that they show signs of comorbid anxiety and depression (Menalled et al. 2009; Pouladi et al. 2009). Few mouse models show a similar behavioral phenotype and there are no existing mouse models that show those symptoms *de novo*. Therefore, there is a potential use of these mouse models for the purpose of investigating comorbid anxiety and depression, for which the neurobiology is poorly understood, partly due to the lack of a valid translational model. These mouse models and HD patients share similar a pathophysiology to healthy patients and knockout mouse models of affective disorders, which makes it a useful tool for discovering new treatments. It is known that the pathology of HD is due to the abnormal mutant huntingtin protein expression and the loss of wildtype huntingtin protein. This protein is present ubiquitously in all individuals but is primarily involved in the regulation of neurotransmission in mature adults (Saudou and Humbert 2016). The mutant protein affects neurotransmission in HD patients, which is also impaired in patients with Major Depressive Disorder and Generalized Anxiety (Pla et al. 2014; Hamon and Blier 2013). Therefore, this report provides evidence for the first *de novo* model cormorbid anxiety and depression. It is the hope that the validation this mouse model will facilitate additional studies into the investigation of the etiology of neuroaffective disorders

.

6.7 Future directions

Neuronal loss in different subdivisions of the DRN and MRN is associated with affective disorder. Several studies have elucidated the involvement of different subdivisions of the DRN and MRN in affective disorders (Baumann et al. 2002; Lowry et al. 2008; Calizo et al. 2011; Spiacci et al. 2012; Paul and Lowry 2013). These subdivisions were determined by the differential rhombomeric origin of the neurons during development (Alonso et al. 2012) as well as their distinct projections to forebrain structures (Muzerelle et al. 2014), which has been to used to show that these subdivisions are functionally distinct. The major subdivisions of the DRN are the dorsal, ventral, caudal, and lateral subdivision (Muzerelle et al. 2014). The MRN can be divided into a rostral and caudal subdivision (Alonso et al. 2012). In patients with MDD, there was evidence of neuronal loss in the ventral subdivision of the dorsal raphe but not the dorsal or caudal subdivision (Baumann et al. 2002). In animal models, anxiogenic drugs and stressful stimuli activate serotonergic neurons in the dorsal and caudal subdivision, which implicates this region in anxiety and depression respectively (Paul and Lowry 2013). In animal models, the MRN was implicated in an avoidance response to stressful stimuli, which implicates this structure in depression-related behavior (Paul and Lowry 2013).

It would be beneficial to assess if selective loss of serotonergic neurons in the distinct raphe subnuclei is evident in the BACHD mouse model or other HD mouse models. This would enable a better understanding of the pre or postsynaptic origin of serotonergic deficits in affective disorders. These studies are also necessary to determine if the reduced serotonin efflux in the BACHD mouse model is a result of loss of

serotonergic neurons. It remains unknown if this is a pathological feature of HD. This can be investigated by conducting unbiased stereological counting of tryptophan hydroxylase-immunostained serotonergic neurons in the raphe (Altunkaynak et al. 2012; (Bang et al. 2012).

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