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Escitalopram Treatment to the HIV-1 Transgenic Rat: Spine Dynamics, Telomere Quantification and Neurogenesis

Adam Ray Denton

ESCITALOPRAM TREATMENT TO THE HIV-1 TRANSGENIC RAT: SPINE DYNAMICS, TELOMERE QUANTIFICATION AND NEUROGENESIS

by

Adam Ray Denton

Bachelor of Science
East Tennessee State University, 2016

Master of Arts
University of South Carolina, 2019

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Accepted by:

Rosemarie Booze, Major Professor

Charles Mactutus, Committee Member

Steven Harrod, Committee Member

Edsel Pena, Committee Member

Tracy L. Weldon, Interim Vice Provost and Dean of the Graduate School

DEDICATION

I dedicate this dissertation first and foremost to my parents, Gary and Elizabeth. Without their unconditional support and kind words, I would have never made it to this point in my life. Through their consistent support, I was able to follow my decision to become the first member of my family to pursue a Ph.D. and an academic path. Next, I dedicate this dissertation to my core group of friends in Virginia and Tennessee. Throughout the mini-vacations, late-night antics, and memorable moments, you kept me motivated and driven while never letting me forget to have a laugh and a good time. Finally, I dedicate this dissertation to my cat, Inky.

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ABSTRACT

HIV is a serious viral infection that persists in the brain despite treatment. Approximately half of all seropositive patients will experience some degree of comorbid depression, as well as HIV associated neurocognitive disorders. The present research sought to evaluate the therapeutic efficacy of escitalopram, a selective serotonin reuptake inhibitor in ameliorating neuroanatomical and biochemical markers associated with HIV infection. The central purpose of this research is to characterize the effects of escitalopram treatment upon dendritic spine proliferation in the nucleus accumbens. Previous research has consistently demonstrated impaired synaptodendritic integrity in this region, with underlying mechanisms remaining unclear. A secondary focus of the proposed research was to quantify telomere length in the HIV-1 transgenic rat treated with escitalopram. Finally, neurogenesis in the hippocampus was examined using immunohistochemical methods. Escitalopram successfully reversed HIV-1 mediated synaptodendritic damage in the nucleus accumbens. HIV-1 rats treated with escitalopram exhibited population shifts in spine morphology and increased dendritic branching. However, escitalopram did not appear to be successful at promoting neurogenesis and likewise did not produce an increase in telomere length as quantified by qPCR. Collectively, these findings illustrate the therapeutic potential of escitalopram in treating HIV-1 associated synaptic damage but suggest that further research in the potential treatment is necessary.

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CHAPTER 1

INTRODUCTION

The human immunodeficiency virus (HIV) is a serious viral infection affecting approximately 37 million people worldwide as of 2017. If untreated, HIV can lead to acquired immunodeficiency syndrome (AIDS) and persists in the body despite treatment (CDC, 2019). The condition is estimated to affect roughly 0.8% of adults ages 15-49 (World Health Organization, 2017). In that same year, approximately 37,000 new cases were estimated to have occurred, which may remain unknown to infected individuals (CDC, 2019; World Health Organization, 2017). 66% of cases are estimated to be transmitted by male to male sexual contact, with heterosexual contact comprising 24% of new HIV diagnoses in 2018 (CDC, 2019). Injection drug use was responsible for 7% of new diagnoses in that same year, with the remaining 3% comprised of some combination of sexual contact and injection drug use (CDC, 2019). In 2017, there were 16,350 deaths among adults and adolescents diagnosed with HIV, though these numbers are not reflective of HIV induced death alone (CDC, 2019).

HIV impedes the immune system with specific detriment to CD4 cell number, thus increasing the likelihood of comorbid infection. These opportunistic infections are benefited by the compromised immune system and are closely associated with AIDS, the last stage of HIV infection (CDC, 2019). HIV was likely first transmitted to humans through chimpanzees in Africa near the end of the 19th century. The simian immunodeficiency virus (SIV) was likely transmitted to humans and underwent mutation

into HIV when humans came into contact with SIV infected chimpanzee blood due to the hunting and consumption of chimpanzees (CDC, 2019).

HIV is transmitted through contact with contaminated bodily fluids. Most commonly, transmission occurs from sexual contact with an infected person, or during needle sharing between drug users (CDC, 2019). HIV has the potential to be transmitted through blood, semen, breast milk, and vaginal or rectal fluids. For infection to occur these fluids must come into contact with damaged tissue or a mucous membrane (CDC, 2019). In the United States, the spread of HIV occurs mainly during sexual intercourse with an HIV seropositive person without the use of prophylactic measures or needle-sharing with an infected individual (CDC, 2019). Less commonly, HIV can be spread from mother to child during pregnancy, birth, or breastfeeding (CDC, 2019). In extremely rare cases, HIV has been reported to be transmitted through bites (Akani et al., 2007) or blood transfusion (Lackritz, 1998).

Untreated HIV infection typically involves three stages of disease progression (CDC, 2019). Within approximately 2-4 weeks following initial infection, flu-like symptoms develop, which can last up to several weeks. This initial response is the body's natural attempt to fight the infection. During acute HIV infection (Stage 1) very high amounts of the virus are present within the bloodstream and individuals are considered very contagious (CDC, 2019). From this initial infection, the virus becomes inactive. This stage is termed clinical latency or asymptomatic HIV. The latency period may last for many years dependent upon treatment adherence. Despite the name, viral transmission is still possible during this phase (CDC, 2019). Due to the decreased reproductive load of the virus, individuals at this stage of disease progression may not present any detectable

symptoms. At the end of this phase, the HIV viral load begins to increase which consequentially drives down CD4 count as the virus progresses into stage 3, Acquired Immunodeficiency Syndrome (CDC, 2019). AIDS is the most severe phase of HIV infection, marked by significantly compromised immune systems often resulting in comorbid infections. Without treatment, the prognosis of AIDS is a life expectancy of around three years (CDC, 2019). AIDS is typically diagnosed when CD4 cell count drops to below 200 cells/mm. At this stage, viral load is high and infection transmission is more probable (CDC, 2019).

The invention and proliferation of combination antiretroviral therapy (cART) has dramatically increased the prospects of living with HIV, although seropositive individuals still suffer from many deficits associated with HIV. With successful adherence to antiretroviral therapy, HIV viral load in the bloodstream can be reduced to undetectable levels, resulting in increased longevity for the patient and decreased likelihood of viral transmission (CDC, 2019). Although cART treatment has the potential to reduce the viral load in the periphery, the virus persists in the central nervous system (Ellis et al., 2008). Most relevant to the present discussion, conditions such as clinical depression and apathy are two such conditions that persist in HIV seropositive individuals despite cART treatment.

Approximately half of all HIV-infected persons will develop some degree of clinical depression throughout his/her lifetime. (Savetsky et al., 2001; Rabkin et al., 2008; Campos et al., 2010; Bhatia and Munjal, 2014, Castellon et al., 1998). Comorbid depression remains a serious blockade to successful treatment of HIV and its associated conditions (Farinpour et al., 2003), with depression significantly impacting both

adherence to treatment and medical appointment attendance (Horberg et al., 2008; Pence et al., 2018; Yoo-Jeong et al., 2016). In addition, apathy remains a common psychiatric disturbance among HIV infected individuals. The high incidence of comorbidity between apathy and depression is unsurprising given the well-documented relationship between motivational dysregulation and depression as documented by previous research (Castellon, et al., 1998; Marin, Firinciogullari, and Biedrzycki, 1993). In addition to depression and apathy, approximately 50% of seropositive individuals will develop some degree of HIV-associated neurocognitive disorder (HAND) despite adherence to cART (Sanmarti, 2014 Bryant et al., 2015).

The spectrum of HIV-associated neurocognitive disorders (HAND) includes classifications such as asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD) (Clifford and Ances, 2014). The majority of cases of HAND are comprised of ANI and MND, which are typically diagnosed through neuropsychological testing. HAD is most commonly associated with advanced HIV progression (Clifford and Ances, 2014). The clinical characteristics of HAND are commonly classified according to the Frascati Criteria (Sanmarti et al., 2014). ANI involves mild cognitive impairment that is attributed to HIV expression and no other cause such as dementia or delirium. In order to meet the criteria of ANI and not something more severe, the cognitive impairment must not interfere with daily activities, although at least two cognitive areas (e.g. memory, attention, language, processing speed, motor skill) are documented at being greater than one standard deviation below the population average (Sanmart et al., 2014). The criteria for MND is very similar to ANI but involves mild interference in daily functioning related to

neurocognitive impairment. Finally, the most serious component of HAND, HIV-associated dementia, involves a significant interference with daily living and significant cognitive impairment in two of the previously described domains indicated by performance of greater than two standard deviations below the population mean (Sanmarti et al., 2014). This presence of cognitive disturbance further exacerbates the relationship between depression, apathy, and HIV-1 infection.

The development of apathy is attributed directly to the effects of viral infection, specifically to the consequent expression of the neurotoxic viral proteins Tat and gp120 (Bertrand et al., 2018; McIntosh et al., 2015). These neurotoxic proteins produce harmful effects upon the dopaminergic system and the neural circuitry underlying reward pathways (McIntosh et al., 2015). The HIV-1 protein Tat inhibits vesicular monoamine transporter (VMAT2) functioning (Midde et al., 2012) which plays a functional role in packaging dopamine into synaptic vesicles for eventual release (Caudle et al., 2007), This disruption may play a critical role in the underlying dopaminergic pathology that has been documented in the HIV-1 Tg rat in addition to DAT dysfunction (Javadi-Paydar et al.,2017; Denton et al., 2019).

Dopaminergic disruption found during HIV infection has been recorded in primary neuronal cell cultures (Aksenov et al., 2008; Bertrand et al., 2013; Ferris, et al., 2008; Zhu et al., 2009); HIV-1 animal models (Fitting et al., 2015; Javadi-Paydar et al., 2017; Bertrand et al., 2018; Paris et al., 2014; Denton et al., 2019) and human HIV-positive brain tissue (Silvers et al., 2006; Kumar et al., 2011 Purohit et al., 2011). While all of these findings are no doubt important, a discussion of the relationship between dopamine and dendritic spines within HIV would not be complete without critical

reference to the HIV-1 Transgenic (Tg) rat. The HIV-1 Tg rat brain contains 7 of the constituent 9 genes that comprise the HIV viral genome, resulting in a non-infectious, long-term model of HIV-1 viral protein exposure (Reid et al., 2001; Vigorito et al., 2015. The HIV-1 Tg rat was initially generated using an infectious provirus derivation following the deletion of the *Sph1-Bal1* fragment that encompasses the *gag* and *pol* genes of the virus, which renders the HIV-1 Tg rat non-infectious and completely safe for experimental handling (Reid et al., 2001). Production of viral proteins, such as Tat and gp120 proteins, remains under the control of the LTR promoter.

Studies using the HIV-1 Tg rat to examine dopaminergic kinetics have consistently demonstrated impaired release and reuptake of dopamine (Javadi-Paydar et al., 2017; Denton et al., 2019; Denton et al., 2020). Dopaminergic functioning in the HIV-1 Tg rat following long-term HIV-1 protein exposure is characterized by lower peak concentrations, and slower reuptake (Denton et al., 2019), which is consistent with *in-vivo* PET imaging studies of the HIV-1 Tg rat (Sinharay et al. 2017) and findings in human HIV-1 PET imaging studies (Chang et al. 2008). Denton et al (2019), is one of the first studies to examine dopaminergic deficits in the intact HIV-1 Tg rat using an *in-vivo* fast-scan cyclic voltammetry (FSCV) paradigm.

Fast-scan cyclic voltammetry (FSCV) is an electrochemical technique that allows for the rapid detection of a broad range of chemical species. FSCV enjoys a competitive advantage over other available techniques in that concentration of the target analyte is observed in real-time, with quantification of the target analyte occurring rapidly. Moreover, identification of the target analyte is provided via the cyclic voltammogram produced throughout the recording period (Robinson, et. Al, 2003).

FSCV applies a triangular waveform within a target analyte-specific voltage range to an electrode. The resting potential is kept at a voltage level insufficient to oxidize the target analyte, then rapidly increased at a high scan rate to a target voltage to promote oxidation. The potential is then returned to starting potential, thus produce a detectable change in electrical current in the opposite direction. This cycle of oxidation and reduction produces time-resolved peaks that allow for the quantification of the target analyte. Dopamine in the caudate nucleus was the first neurotransmitter quantified by FSCV (Millar et al., 1985). Since its initial inception, FSCV has been used to evaluate dopamine transmission *in vitro, ex vivo,* and *in vivo* (Budygin, et. Al, 2001; Kelly and Wightman, 1987; Millar et al., 1985; Phillips et. Al, 2003; Robinson et. Al, 2003; Troyer and Wightman, 2002).

FSCV provides an indisputable tool for investigating the profound dopaminergic impairment in the HIV-1 Tg rat, and thus for investigating the broad relationship between HIV and dopamine. To this end, Denton et al. (2019) reported a profound reduction in evoked dopamine response in anesthetized HIV-1 Tg rats relative to F344/N controls. In addition to the profound decrease in dopaminergic peak (Cmax), a similar impairment in reuptake of dopamine was found in HIV-1 Tg rats (Denton et al., 2019). These results were since replicated by an additional study using an identical FSCV protocol (Denton et al., 2021). As previously discussed, these findings are in line with clinical findings in HIV seropositive patients, thus illustrating the profound dopaminergic damage present in both animal models of HIV and seropositive patients. Moreover, morphological changes in dendritic branching and dendritic spine morphology likely underlie and exacerbate neurotransmitter pathology in HIV-1.

CHAPTER 2

DOPAMINE, MOTIVATIONAL DYSREGULATION, AND DEPRESSION

The function of dopamine has long been studied using a variety of methods that have contributed to our fundamental understanding of its actions throughout the nervous system. Dopaminergic function has been implicated in motivation, reward-seeking, and motor control in addition to many other functions. Dopaminergic dysfunction has been associated with mood disorders such as depression and the motivational dysregulation observed in addiction (Nestler and Carlezon, 2006), in addition to apathy and anhedonia associated with viral infections such as HIV-1 (Bertrand et al., 2018; Denton et al., 2019). Furthermore, dopaminergic dysfunction and cellular death are associated with movement conditions such as Parkinson's disease and Huntington's disease.

Pathways in the brain that involve dopaminergic transmission include the nigrostriatal pathway, which projects from the substantia nigra to dorsal striatal structures such as the caudate and putamen. This pathway has been implicated in associative learning in addition to reward-related cognition (Ikemoto, 2010). The tuberoinfundibular dopaminergic pathway projects from the arcuate nucleus of the hypothalamus to the pituitary gland where dopamine is released into the median eminence and circulated through the hypophyseal portal system. In this way, dopamine influences the regulation of hormones such as prolactin and plays a role in the endocrine response of the body (Malenka et al., 2009). Other pathways of note include the hypothalamospinal projection

which is critical in motor function and the incertohypothalamic pathway which projects from the zona incerta to the brain stem and is involved in the regulation of visceral activities (Malenka et al., 2009).

While there are several dopaminergic pathways of note throughout the brain that contribute to a wide variety of behavioral functions, the mesocorticolimbic dopaminergic pathway is central to the present discussion. This pathway can further be divided into the mesolimbic pathway which includes dopaminergic projections from the ventral tegmental area to structures such as the nucleus accumbens in the ventral striatum (Ikemoto, 2010). These particular structures in this pathway are critical to the present discussion, as they are involved in reward-related behaviors. Dysfunction in this pathway is associated with a host of neurological conditions, from schizophrenia to depression (Nestler and Carlezon, 2006). Dopamine from the ventral tegmental area is also projected to the prefrontal cortex comprising the mesocortical pathway. This region is further associated with reward-related functions and is associated with executive functioning (Ikemoto, 2010).

A great deal of our current understanding of dopaminergic function in reward comes from early electrical brain stimulations involving dopamine (Olds and Milner, 1954; Wise, 1978). Rats were shown to self-stimulate via electrode implantations in dopaminergic areas of the brain (Wise, 1978). Furthermore, dopaminergic function was consistently implicated in appetitive motivated behaviors (Panksepp, 1971). From these early studies, it was increasingly evident that dopamine played a critical role in pleasure or reward. Later studies would further increase the understanding of dopaminergic function by showing that dopamine was not only released in response to pleasurable,

rewarding events, but also in response to stress and aversive stimuli (Puglisi-Allegra et al., 1991). Thus, dopaminergic function is essential under both negative and positive emotional states and appears to promote general behavioral arousal under both positive and negative conditions (Alcaro et al., 2007). This has led some researchers to suggest homeostatic mechanisms of dopaminergic function, such as the seeking of safety, in addition, to reward (Ikemoto and Panksepp, 1996; Alcaro et al., 2007). Furthermore, tying the discussion to behaviors that are considered to uniquely human, dopamine function is implicated in the development of "personality traits" such as novelty seeking or impulsivity (Bardo et al., 1996; Cardinal et al., 2004) and even to traits such as extraversion (Depue and Collins, 1999).

Dopaminergic functioning is critical in a wide range of processes both behavioral and cognitive. The inherent complexity of dopaminergic functioning and the incredible array of processes that it is involved in presents something of an organizational nightmare when attempting to characterize dopaminergic related functioning. Fortunately, however, the Research Domain Criteria (RdoC framework) provides a considerably useful organizational strategy for organizing such functions. The Research Domain Criteria was proposed by the National Institute of Mental Health in a 2010 discussion published in the American Journal of Psychiatry (Insel et al., 2010). This model was proposed in response to concerns raised over the Diagnostical and Statistical Manual of Mental Disorders (DSM) published by the American Psychological Association, and the International Classification of Diseases published by the World Health Organization. While it is acknowledged that both the DSM and ICD have indeed facilitated reliable and valid clinical research and possess some diagnostic power, they are not without fault, however.

Such diagnostic categories based upon clinical practice often fail to align with findings from neuroscience and genetics (Insel et al., 2010). Furthermore, these categories are not predictive of clinical responses to treatment. Most grievously, however, these categories are described wholly based upon potential signs and symptoms that characterize disorders but offer little discussion as to the underlying mechanisms of dysfunction. Indeed, even a cursory glance through the DSM or ICD will yield these observations. Conditions are described by virtue of the clinical symptoms they typically present. No discussion is presented as to specific genetic dysfunction, cellular and biochemical mechanisms, or underlying pathologies. While treatment schemes such as those used by the DSM or ICD undoubtedly have their place in clinical practice and are immensely helpful in describing signs and symptoms of conditions, the organizational framework does not lend itself to encapsulating the often rapid findings generated by research and are often inadequate when discussion biological mechanisms that underlie these conditions. As a moment of caveat, however, the DSM will be cited in the present discussion as it has significant utility in describing observed clinical descriptions which are essential for setting up the present discussion of pathological mechanisms. However, in terms of research, the RdoC is useful for linking clinical findings to observed pathology and provides an essential organizational framework as we will see in the coming discussion.

Another concern raised by Insel et al. (2010) in arguing for the necessity of the RdoC is the observation that significant problems often arise when only descriptive diagnostic systems are designed without an understanding of pathology. Specifically, disorders that were once considered to be the same based upon clinical presentation are markedly heterogeneous when examined in the laboratory. An example of this

phenomenon cited is the distinction between the destruction of pancreatic cells versus insulin resistance that characterizes distinct forms of diabetes mellitus (Insel et al., 2010). Based solely upon diagnostic criteria, the forms of diabetes appear to be clinically unitary as they present with similar symptoms, but even a casual test in the laboratory confirms the existence of divergent pathologies. To extend this argument posed by Insel et al., (2010), the inverse to the example presented is also true. Clinical manifestations that appear too disparate (as is the case with many of the pathologies to be discussed presently) are often clustered into distinct categories. This forced categorization often ignores similarities in pathology that may underlie the conditions. Thus, the RdoC was born from the desire to achieve an approach to classification that involves not only clinical descriptions but also incorporates findings from laboratory studies and genomic studies. In this way, the RdoC seeks to reconcile clinical and laboratory findings and produce a classification scheme that has utility in both diagnosis and research.

To achieve these somewhat lofty goals, the RdoC framework rests upon three critical assumptions. The first is that mental illnesses are brain disorders. In contrast to more neurological disorders that can be associated with identifiable brain lesions or injury, mental disorders can be characterized by disorder and dysfunction of brain circuitry (Insel et al., 2010). This assumption is critical for the present discussion, as conditions such as HIV and depression have considerable impairments in brain circuit function. The second assumption of the RdoC framework hinges upon the first and states that dysfunction in neural circuitry that leads to brain disorders can readily be identified with the tools of clinical and experimental neuroscience. Tools such as functional imaging, electrophysiology, and anatomical analysis can provide considerable insight

into the world of brain function and dysfunction. This second assumption is also critical to the present discussion, as much of the circuit dysregulation that will be discussed involves methods typically used to quantify dendritic spines such as confocal and electron microscopy, immunohistochemical methods such as modified Golgi staining, and techniques from brain imaging. In addition, quantitative methods such as Sholl analysis and computational neuroscience aid the understanding of brain dysfunction across the conditions that will be discussed.

The third and final assumption of the RdoC framework is that data extracted from genetic and neuroscientific studies can produce biosignatures that will augment the understanding of clinical signs and symptoms, and will produce updated frameworks for clinical practice (Insel et al., 2010). Indeed, this third assumption represents a goal of this dissertation that will be addressed in the concluding paragraphs. Across both HIV and depression, certain trends or biosignatures can be observed that provide critical insight into similar underlying pathologies present. In this way, potentially novel treatment approaches can be developed and discussion may move beyond classifying these conditions as discreet and separate conditions.

Dopamine is considered a molecule under the framework of the RdoC and is associated with the following constructs: negative valence systems, positive valence systems, cognitive systems, social processes, arousal and regulatory systems, and sensorimotor systems (NIMH, 2020). While the crux of the discussion in this chapter is related primarily to negative and positive valence systems, it is helpful for the present discussion to mention, at least in brief, some of the other constructs associated with dopamine. Under the domain of cognitive systems, dopamine is further associated with

reward-related cognitive systems. First and arguable foremost are the constructs of attention and working memory, which are undeniably critical in the presentation of and response to rewarding stimuli. The construct of working memory can further be divided into subconstructs that describe working memory maintenance, capacity, interference control, and updating. The final construct worth mentioning that associates dopamine with cognitive systems is cognitive control (NIMH, 2020). This construct can further be divided into goal selection, response selection, and performance monitoring (NIMH, 2020). Goal selection as it relates to motivated behavior involves the selective updating and maintenance of goal-driven motivated behavior, in addition to associated cognitive representations.

The final three dopamine-related constructs in the RdoC are of less importance to the present discussion but deserve brief mention nonetheless. Dopamine as it relates to social processes involves the constructs of affiliation and attachment in addition to social communication, in particular, the reception of facial communication (NIMH, 2020). Under the domain of arousal and regulatory systems, dopamine is further associated with biorhythms in addition to the fundamental construct of arousal, which was discussed previously. Finally, dopamine's involvement in sensorimotor systems includes the constructs of motor action and habit formation (NIMH. 2020).

Central to the present discussion, however, is dopaminergic involvement in the constructs of negative and positive valence systems, particularly as it relates to apathy and anhedonia. As described in the previous chapter, rates of comorbid HIV and depression are significantly high. Underlying both of these conditions are persistent apathetic and anhedonic symptoms. HIV comorbid depression involves significant

alterations to both negative and positive valence systems that underlie clinical features such as anhedonia in addition to impairments in reward learning and valuation. Under the domain of negative valence systems, two further constructs are discussed in the RdoC. The first, acute threat, is described as the defensive motivational system to promote behaviors that protect the organism from danger, either real or perceived (NIMH, 2020). Furthermore, acute threat, or "fear" involves adaptive responses to conditioned or unconditioned threat stimuli (NIMH, 2020). The second construct under this domain is frustrative non-reward, which encompasses reactions elicited in response to either the presentation or withdrawal of a reward by the inability to obtain positive rewards following sustained effort (NIMH, 2020). Anhedonia, which is a critical concept in the present discussion, falls under the umbrella of negative valence systems and will be discussed in more detail with respect to the neuropathology of dendritic spines and dopamine.

Continuing with the present discussion of dopaminergic function, reward learning is the process by which an organism acquires information that predicts positive outcomes of stimuli or contexts (NIMH, 2020). This construct further includes the subconstruct of reward prediction error, which can be positive in the case of reward being greater than expected, or negative in the case of a smaller than expected reward (NIMH, 2020). Finally, dopamine is critically involved in the construct of reward valuation, which is the process by which the probability of a rewarding outcome is computed (NIMH, 2020). This outcome is computed based on information that the organism possesses that was acquired through learning, and further includes the notion of effort.

While positive and negative valence systems are separated in the RdoC framework for ease of organization, it is important to note that these systems are inextricably intertwined. Each of these systems functions together to produce an organism that is optimally suited for each particular environment. Deficits in one valence system are very likely to produce consequent deficits in the other. Clinically speaking, the dramatic interplay between these two systems and the effects of impairment produce many of the phenotypes typically observed across conditions with associated deficits in reward processing such as depression, schizophrenia, and HIV-associated apathy and neurocognitive disorders. Furthermore, each of these conditions is marked by significant impairments in dopaminergic functioning and associated pathology of dendritic spine morphology and populations. Now that the groundwork for understanding the function of dopamine in the brain has been established, attention can now be turned to apathy and anhedonia, which are critical symptoms that need to be discussed with respect to HIV and comorbid depression.

Prior to discussing the specific role of dendritic spines in HIV and its associated symptomologies, it is important to define some important concepts that arise out of aberrations in valence systems. Two such phenomena that will be central to the present discussion are apathy and anhedonia. As will be discussed, these conditions are central phenotypic features in a host of neurological and psychological conditions. Indeed, apathy and anhedonia are common features that cut across traditional diagnostic lines consistently present in cases of HIV, depression, and drug addiction. Moreover, these conditions often present significant impairments to the successful treatment of these conditions.

Both apathy and anhedonia involve the degradation of motivational processes that guide goal-directed behavior. In terms of RdoC constructs, both of these conditions are centrally related to both positive and negative valence systems, and likely involve a central disfunction in both. More specifically, these conditions cut across constructs such as loss, reward valuation, reward effort, effort valuation, reward prediction, and reward response (Thant and Yager, 2019; NIMH 2020.) However, fundamental mechanisms of both apathy and anhedonia are not well elucidated, and defining potentially distinct disorders of motivation requires significantly more research, including predictive validators, pathogenesis, diagnostic methods, and treatments (Thant and Yager, 2019).

Furthermore, it is unclear if there is a meaningful distinction to be found between apathy and anhedonia as they involve many of the same motivational dysregulations and are present in many of the same conditions. These similarities have led many researchers to posit that they may thus have similar, if not the same, underlying pathogenesis (Husain and Roiser, 2018). Both involve significant alterations and impediments to normal motivational functioning and are typically associated with mesocorticolimbic dysfunction (Epstein and Silbersweig, 2016). Moreover, both are often comorbid across a wide range of conditions that are associated with dopaminergic pathology (Epstein and Silbersweig, 2016). There remain significant knowledge gaps within the literature surround the nature of both apathy and anhedonia, which is reflected in the clinical treatment of these conditions, often in the context of treatment for comorbid conditions. To date, no demonstrably effective therapeutics exist for the treatment of apathy and anhedonia (Husain and Roiser, 2018).

While the relationship between apathy and anhedonia may remain somewhat unclear, it might be useful to unpack some of the more prominent clinical descriptions of the conditions. Apathy is fundamentally a disorder of motivation that is typically characterized by a reduction in initiation of goal-directed behaviors (Ang et al., 2017). While apathy is indeed a common comorbidity among many neuropsychological disturbances, apathy may also manifest itself in otherwise healthy people with no history of psychological or neurological disturbance (Ang et al., 2017). Furthermore, it has been suggested that apathy is not simply the unitary lack of motivation to engage in behavior but is a complex multidimensional construct that spans motivation across, cognitive, emotional, and behavioral domains (Levy and Dubois, 2006; Ang et al., 2017). This idea is corroborated by survey studies employing factor analysis to examine latent variable structures across several more well document apathy inventories developed for clinical practice. Ang et al., (2017) administered the Lille Apathy Rating Scale (LARS) to a sample of 505 clinically healthy individuals and elucidated a three-factor structure now termed the Apathy Motivation Index (AMI). The AMI was produced by an exploratory factor analysis with promax factor rotation of the LARS. Psychometric properties of the AMI were then confirmed a separate via confirmatory factor analysis across a separate sample of more than 400 clinically healthy individuals (Ang et al., 2017). Three domains of apathy were thus identified: behavioral activation, emotional sensitivity, and social motivation. Behavioral activation is defined as the tendency to self-initiate goal-directed behavior and is further associated with the initiating and maintaining of task-relevant responses (Ang et al., 2017). Emotional sensitivity is a measure of the individual's feelings of positive and negative affect. Interestingly, there was not a statistically

significant correlation between emotional sensitivity and behavioral activation, suggesting a distinction between the behavioral and emotional aspects of apathy (Ang et al., 2017). The final factor, social motivation examines the individual's engagement in social interaction, which was significantly positively correlated with both behavioral activation and emotional sensitivity (Ang et al., 2017).

Perhaps unsurprisingly, findings from the AMI are heavily correlated to measures of anhedonia such as the Snaith-Hamilton Pleasure Scale (SHAPS). Anhedonia is classically defined as the lack of interest or pleasure in or derived from normally rewarding activities. Historically, anhedonia has been associated with "pleasure" and "liking", but more recent evidence suggests that in addition to these constructs, anhedonia involves further constructs such as "wanting" (Epstein and Silbersweig, 2015). The term "anhedonia" is most consistently used when referring to depression or schizophrenia, though evidence has shown that the construct is associated with several other conditions, and, similarly to apathy, can be present to some degree in the healthy population (Epstein and Silbersweig, 2015). The lack of emotional feedback from typically rewarding experiences is particularly detrimental to motivational processes, as positive reinforcement is critically necessary for maintaining goal-directed behavior (Everitt and Robbins, 2005). All subscales on the AMI were found to be significantly highly correlated to scores on the SHAPS, suggesting a fundamentally close relationship between apathy and anhedonia (Ang et al., 2017). The measures were not perfectly correlated, however, suggesting some distinction between apathy and anhedonia (Ang et al., 2017). However, given the close association between apathy and anhedonia, it is

unsurprising to learn that both typically present themselves as clinical manifestations of both HIV and clinical depression.

Depression is one of the most common mental illnesses worldwide, with an estimated 264 million people affected globally (WHO, 2020). Depression is a distinct condition from the occurrence of usual emotional fluctuation. Depression, especially when persistent and severe, can lead to extreme impairment in the quality of life for those affected and individuals around them (WHO, 2019). Serious consequences arise for individuals suffering from depression, who often experience a severe disruption in interpersonal function in both professional and social lives (NIMH, 2020; WHO, 2020). Perhaps the most serious consequence of depression is suicide, which is the second leading cause of death in individuals between the ages of 15 and 29, and claims nearly 800,000 lives each year (WHO, 2020). Most alarmingly, depression is on the increase globally. According to the World Health Organization, the number of individuals suffering from depression worldwide has increased by 50% between the years of 1990 and 2013, with estimated cases increasing from approximately 416 million to 615 million between these years (WHO, 2016). Currently, it is estimated that as many as one in five individuals are affected by depression, with a cost to the global economy of approximately 1 trillion dollars each year investing in possible treatment opportunities.

Depression results from an incredibly complex interaction between psychological and biological factors with much of the complexity underlying the condition still unknown (WHO, 2019). Significant risk factors for depression include genetics, adverse life events (e.g. trauma or bereavement), and social factors (WHO, 2020). The onset of depression can begin at any age, with earlier onset of depression in adolescents becoming

more prevalent (NIMH, 2020). In older adults, depression is often comorbid with other conditions that present a significant detriment to both patient quality of life and potential disease outcomes, such as HIV. With the increasing global burden of depression and related conditions, significant headway has been made in advancing treatment, although many blockades to successful treatment still exist and continue to be a source of frustration for scientists and clinicians alike.

The role of the serotonergic and noradrenergic systems has been consistently explored with respect to depression. All successful therapeutics for depression in some way alter these pathways (Nestler and Carlezon, 2006). However, the importance and centrality of dopamine to the manifestations of the condition cannot be ignored. Studies from the field of drug addiction and reward have consistently implicated the dopaminergic pathway from the ventral tegmental area of the midbrain to the nucleus accumbens as playing a key role in reward both for drugs and natural rewards (Koob and Le Moal, 2001; Nestler and Carlezon, 2006). Both of these areas receive strong excitatory inputs from the frontal cortex, hippocampus, and amygdala. Each of these areas, in turn, receives dopaminergic input from the ventral tegmental area (Nestler and Carlezon, 2006). This dopaminergic circuitry is key for reward motivation and reward-seeking. Given that anhedonia is often the major reported disturbance in cases of depression, this dopaminergic circuitry is a prime target for dysfunction (Nestler and Carlezon, 2006). The idea that dopaminergic dysfunction of this pathway mediates depressive behavior was proposed based on studies with dopamine antagonists (Wise, 1982). Since that time, much more research has demonstrated the key role of dopamine in anhedonic behavior.

Anhedonia remains one of the toughest symptoms of depression to treat. Second-generation antidepressants, while showing a great affinity for the serotonin system, have considerable difficulty in treating anhedonia (Belujon and Grace, 2017). Antidepressants, in particular SSRI's, have shown limited success in repairing motivation and reward-related cognition (Belujon and Grace, 2017). Furthermore, the persistence of anhedonia is a major impediment to the successful treatment of depression and suggested to play a role in relapse in cases of drug abuse (Koob and Le Moal, 2001; Belujon and Grace, 2017). Anhedonia, in the context of clinical depression, often encompasses more than just the inability to experience pleasure. The dopaminergic circuitry of the ventral tegmental area and nucleus accumbens mediates reward prediction, reward motivation, and reward responsiveness (Belujon and Grace, 2017). This disruption in reward-motivated behavior is more characteristic of clinical depression and is consistent with clinical descriptions of individuals suffering from major depressive disorder (Sherdell et al., 2012).

Anhedonia in depression is associated with reduced dopaminergic response to reward, lower dopamine transporter binding affinity, and downregulation of dopaminergic function (Belujon and Grace, 2017). Moreover, studies have shown increased D2 and D3 receptor binding in the amygdala, further suggested profound dopamine dysfunction (Belujon and Grace, 2017). These findings have been further replicated in animal models of depression, with altered mesolimbic dopaminergic tone found in both learned helplessness and chronic mild stress models of depression (Dziedzick-Waslewska et al., 1997; Kram et al., 2002; Belujon and Grace, 2017). In addition, lesions to the VTA in rats have been shown to produce increased anhedonic behavior in motivational tasks (Winter et al., 2007). More recent work has shown that

selective inhibition of dopaminergic neurons in the ventral tegmental area leads to depressive phenotypes that are reversible with activation of the neurons (Tye et al., 2013).

As briefly described earlier, disruption in dopaminergic circuitry in clinically depressed patients has serious implications for the successful treatment of depression and anhedonia. The majority of clinically depressed patients receiving medication such as SSRIs do not obtain remission from depression, despite medication adherence (Belujon and Grace, 2017). While there have been mixed findings surrounding the effects of SSRI medication upon dopaminergic functioning, most antidepressants have appeared to reduce dopaminergic firing in the ventral tegmental area (Belujon and Grace, 2017). Moreover, certain SSRIs have been shown to dramatically reduce the firing rate and bursting spikes of dopamine neurons (Belujon and Grace, 2017). As some researchers have begun to speculate, this decrease in dopaminergic function as a result of antidepressant treatment may underlie many of the treatment-resistant aspects of depression. Given the well-established role of dopamine in hedonic and reward processes, decreases in dopaminergic function as a consequence of antidepressant treatment may greatly contribute to antidepressant resistance among patients with depression (Belujon and Grace, 2017). Many clinicians have thus turned to augmentation strategies to prevent antidepressant resistance. The majority of these strategies involve increasing dopaminergic tone as a supplement to SSRI treatment in an attempt to stabilize dopaminergic function and thereby optimize treatment (Belujon and Grace, 2017). The addition of antipsychotic medications to SSRI treatment regimens has shown promise in the treatment of depression. The Food and Drug Administration has approved several

atypical antipsychotics as supplements for SSRI treatment. Atypical antipsychotics have been shown to potentiate the effect of antidepressants such as citalopram and fluoxetine (Kamei et al., 2008; Bourin et al., 2009; Belujon and Grace, 2017). While the specific mechanisms behind the success of these treatments remain elusive, Belujon and Grace (2017) suggest that this mechanism may be homeostatic compensation of dopamine systems such as upregulation or increased activity of tyrosine hydroxylase (Belujon and Grace, 2017).

Further suggesting a role of the mesolimbic dopaminergic pathway in depression, the dopamine D1-D2 heteromer has been extensively studied with respect to depression and anhedonia. While much of the research surrounding depression has been constrained to the prefrontal cortex and hippocampus, research is consistently revealing important activation changes in the mesolimbic pathway, particularly the nucleus accumbens. Preliminary studies indicated that activity in this region is reduced in depressed patients, possibly by increased GABAergic inhibition in the area (Tye et al., 2013). Brain-derived neurotropic factor has been implicated to be a key player in protein expression associated with depression, as many proteins identified that are associated with depression are downstream of BDNF signaling in the nucleus accumbens (Shen et al., 2015). The dopamine D1-D2 receptor heteromer is a dopaminergic receptor complex that is mostly expressed in the nucleus accumbens and has been shown to increase the expression of GABAergic producing enzymes (Shen et al., 2015). This increased production of GABA likely works to produce inhibition in the nucleus accumbens (Perreault et al., 2012; Shen et al., 2015). Moreover, this heteromer has been shown to increase the expression of BDNF signaling in the nucleus accumbens through the action of tropomyosin receptor

kinase B (Hasbi et al., 2009; Shen et al., 2015). Further suggesting a role for the D1-D2 heteromer in the pathogenesis of depression, peptides that disrupt the function of the heteromer have been shown to display antidepressant activity in rats (Shen et al., 2015). Studies examining the effects of pharmacological stimulation of the D1-D2 heteromer with agonists such as SKF 83959 have produced impairment on many behavioral tasks such as the forced swim test and elevated plus-maze in rats (Shen et al., 2015).

Depression and anhedonia, or the disruption in reward processes, has conventionally been associated with pathological disruptions in serotonin and norepinephrine in brain regions associated with memory and cognition. However, an increasing body of research is showing that disruption of the mesolimbic pathway, particularly the nucleus accumbens, underlies dysfunction in reward typically associated with the term “anhedonia”. Disruptions in dopaminergic transmission and increases in GABAergic inhibition of dopamine in the nucleus accumbens are two common findings associated with depression. Thus, the pathological alterations underlying depression are much more complicated than what was once thought. Dendritic spine modification in depression is equally as complicated, with both region-dependent increases and decreases in dendritic spine numbers being reported in the literature (Qiao et al., 2016).

As previously described in Chapter 1, HIV and clinical depression occur together in approximately 50% of the HIV seropositive population (Rabkin et al., 2008). In fact, depression and apathy and are among the most common comorbidities of both HIV and HAND (Cysique and Brew, 2019). Two of the most important factors in the treatment of HIV are treatment adherence and medical appointment attendance. Successful adherence to cART and proactive involvement in medical appointments are crucial steps for HIV

seropositive individuals to take to avoid the progression to AIDS. Comorbid depression with HIV has been demonstrated to significantly reduce both treatment adherence and medical appointment attendance which have to consequence of rendering treatments for HIV less effective and potentially jeopardizing the life and well- being of the patient (Horberg et al., 2008; Pence et al., 2018; Yoo-Jeong et al., 2016). Moreover, these individuals are more than five times likely to commit suicide than HIV seronegative counterparts (Veterans Affairs Administration, 2009). While many studies have been published that document the relationship between HIV and clinical depression in treatment settings, alarmingly few studies have directly evaluated the efficacy of antidepressant medication within the presence of HIV. Moreover, few studies have recognized the similarities in dendritic spine dysfunction between HIV and depression. HIV, depression, and many other conditions that present with disruptions such as apathy and anhedonia have common disruptions in dendritic spine populations, particularly in the nucleus accumbens and the prefrontal cortex.

CHAPTER 3

DENDRITIC SPINES

Dendritic spines comprise essential synaptic structures that are critical for the maintenance of synaptic plasticity (Yuste et al., 2010). Nearly all excitatory synapses in the mammalian forebrain are formed as a result of these structures (Herms and Dorostkar, 2016; Yuste, 2010). Dendritic spines emerge from dendritic shafts, which are protrusions on the dendritic branches of neurons that produce the tree-like arborization of neurons. Dendritic spines can be further characterized by their ultrastructure. Spines are divided into two physiologically relevant sections: spine neck and spine head (Yuste, 2010). The spine head is important in synaptic communication and consists of the presynaptic terminal and postsynaptic density (Yuste, 2010). Spines are generally less than 3 um in total length, from neck to head, and possess a spherical head attached to a more narrow neck (Yuste, 2010).

Dendritic spines can be appropriately categorized based upon their morphology. The most widely accepted classification was proposed by Peters and Kaiserman-Abramof in 1970 and divides spines into three rough categories based upon their morphological characteristics (Peters & Kaiserman-Abramof, 1970; Yuste, 2010). Thin spines are among the most common spine types observed. These spines possess a characteristically thin neck with a head that displays a slightly bulbous appearance. In the adult cortex and hippocampus, approximately 65% of dendritic spine populations are comprised of thin spines (Peters & Kaiserman-Abramof, 1970; Berry & Nedivi, 2017). Owing to their

smaller size, thin spines contain fewer excitatory synapses and are characterized by incompletely developed synaptic integrity (Yuste, 2010; Berry & Nedivi, 2017). Some authors have suggested that these spines are indicative of the capacity for neuronal plasticity, however, owing to the incomplete formation of thin spine synapses (Berry & Nedivi, 2017). Consequently, thin spines have been postulated to play a role in the ability to learn (Berry & Nedivi, 2017). Thin spine populations have been shown to rapidly decrease along the aging process and to deteriorate in a manner directly proportional to cognitive aging (Dumitriu et al., 2010). Consequently, manipulation of thin spine populations is associated with restoration of plasticity in animal models (Hao et al., 2006).

A second category of dendritic spine subtypes is stubby spines. These spines are far shorter than other morphologies and lack a distinctive differentiation between spine head and spine neck, leading to a "squat" appearance (Yuste, 2010.) These spines are characteristic of incomplete synaptic development and are often found during development, although stubby spine populations are maintained in adulthood though in relative scarcity (Yuste, 2010; Berry & Nedivi, 2017). Given the association with these spine types and early development, these structures within the context of pathology are typically viewed as immature. As will be discussed shortly, spine head size has very important implications for synaptic transmission. The relatively small size of these spines and lack of defined spine neck lead to decreased synaptic transmission (Yuste et al., 2010). Increases in stubby spine populations as we will see are often associated with neuropathology, as is the case with clinical depression (Buyukdura et al., 2013).

The third formally recognized category of dendritic spine types is mushroom spines, which earn their namesake due to their distinct appearance. These spines are characterized by their large, bulbous spine head which is clearly distinguishable from the spine neck (Yuste et al., 2010). These spine types are thought to make up greater than 25% of the total mammalian cortex spine volume (Berry & Nedivi, 2017). Mushroom spines represent adult, mature synapses and are important in synaptic transmission owing to the relatively large surface area of the spine head, and thus the capacity for excitatory input. These larger, mushroom spine types are hypothesized to represent the upper limits of synaptic strength and plasticity capabilities (Berry & Nedivi, 2017). Consequently, these mature spines are typically hypothesized to hold little room for further synaptic improvement and are indicative of a mature nervous system (Yuste, 2010; Berry & Nedivi, 2017). Similar to how thin spines are associated with learning or the capacity to learn, mushroom spines are associated with memory and the aftereffects of learning (Berry & Nedivi, 2017). Larger, mushroom spines have greater structural components, owing to their morphology. Namely, these spines are characterized by smoother endoplasmic reticulums and polyribosomes, greater contact with glial cells, and more excitatory receptors. Mushroom spines additionally have more complex postsynaptic densities, while less complex spines such as thin spines have smaller, more macular postsynaptic densities (Yuste, 2010).

A fourth "category" of dendritic spine types that deserves some discussion here is dendritic filopodia, which are typically longer and thinner with no clear spine head (Yuste, 2010). These structures are often described as thin, hair-like appendages on dendrites. Dendritic filopodia are considered to be immature spine types and account for

less than 10% of the total dendritic spine volume in the adult brain (Berry & Nedivi, 2017). Filopodia lack a clear postsynaptic density and are characterized by small synaptic clefts and very few synaptic vesicles (Berry & Nedivi, 2017). Given their lack of synaptic connectivity and relative immaturity, several authors have argued that they should be excluded from studies of spine density. However, as a caveat to this, many additional authors have suggested that this omission is not a trivial task, as it is often difficult to distinguish between the morphology of thin spines and dendritic filopodia without the aid of advanced techniques such as electron microscopy (Berry & Nedivi, 2017).

Though contention exists as to whether or not filopodia should be considered among dendritic spine population counts, dendritic filopodia are not without function in the nervous system, though there is significant disagreement and lack of research (Yuste, 2010). Dendritic filopodia have been hypothesized to play a significant role in spinogenesis, particularly in pyramidal cells of the cortex and Purkinje cells of the cerebellum (Yuste, 2010). Filopodia do not contain a spine apparatus or a smooth endoplasmic reticulum. Smooth endoplasmic reticulums are critical in maintaining calcium transmission, which is a necessary prerequisite for neuronal communication. Smoother endoplasmic reticulums are highly characteristic of mushroom spine types, and play a critical role in dendritic transmission and plasticity (Yuste, 2010). Furthermore, filopodia are comprised of a homogeneous cytoplasm that consists of exclusively actin filaments (Yuste, 2010). This composition makes dendritic filopodia considerably difficult to study, as filopodia exhibit poor impregnations with Golgi staining methods,

and react poorly to fixatives. Consequently, filopodia are seen at considerably lower densities in histological preparations than in *in-vivo* imaging (Yuste, 2010).

Dendritic filopodia share many morphological similarities with other filopodia in the nervous system. Indeed, morphological similarities between dendritic filopodia, axonal growth cone filopodia, muscle filopodia, and glial filopodia are quite striking. Additionally, these filopodia are nearly identical to nonneuronal filopodia. Further suggesting a role of dendritic filopodia in spinogenesis, it has been proposed that the elongated shape of these structures lends itself well to an exploratory function within the extracellular environment. Moreover, these structures are considerably less observed in mature samples, further indicating a potential role in nervous system development (Yuste, 2010). These observations have even led to the formation of a filopodial model of spinogenesis, particularly in pyramidal neurons (Yuste, 2010). Filopodia are observed to occur in groups on dendrites of the neocortex and emerge from potentially critical locations for dendritic development- a feature which further mimics the actions of axonal filopodia and other developing structures (Yuste, 2010). These observations have led to the proposition of "protospines" under this model of spinogenesis. Protospines are described as intermediate dendritic structures emerging from transient filopodia that have become stabilized (Yuste, 2010). Given the transient nature of filopodia, those filopodia capable of locating a viable axon terminal become stabilized and create a "virtual dendrite". This virtual dendrite serves as a location around the perimeter of the existing dendrites which can be explored by the developing neuron in an effort to establish axonal contact (Yuste, 2010). These potential functions of filopodia were described as early as Peters and Kaiserman- Abramof's seminal paper in 1970, and have been independently

proposed by many other researchers (Peters & Kaiserman- Abramof, 1970, Yuste, 2010). Furthermore, the composition of actin that is found in the cytoplasm of filopodia has been proposed to drive dendritic connections that ultimately produce a dendritic spine (Yuste, 2010). There are compelling lines of evidence both for and against this model of spinogenesis, however, with much work needed to further confirm or discredit these ideas (Yuste, 2010).

Several important correlations exist between dendritic spine morphology and synaptic strength. First and foremost, spine head volume in addition to total spine volume is strongly positively correlated with the size of the post-synaptic density. Increased post-synaptic density is furthermore correlated with the number of presynaptic vesicles and the size of the active pre-synaptic zone (Yuste, 2010). Spine morphologies with larger head volumes are correlated with an increased pool of docked synaptic vesicles which in turn is correlated with an increased potential for neurotransmitter release (Yuste, 2010). On the postsynaptic side, the increase in the post-synaptic density associated with larger spines is related to the number of postsynaptic receptors (Yuste, 2010). These findings explain why larger, more mature types of spines such as mushroom spines are associated with increased synaptic strength and capability (Berry & Nedivi, 2017).

Extending our discussion of the morphological features of dendritic spines, it is interesting to note that spine neck diameter or length does not appear to be correlated with the size of the post-synaptic density, or the capacity for neurotransmission (Yuste, 2010). The exception to this is found on apical dendritic trees in pyramidal cells, where spine neck diameter is positively associated with head volume and thus pre-synaptic vesicle counts and post-synaptic density (Yuste, 2010). However, in most cell types, the

regulation of the size of the spine neck and head appear to be differentially regulated (Yuste, 2010).

Given the diverse variation in morphology of dendritic spines, it is reasonable to suppose that morphology has direct consequences on the size of the synapse and synaptic connectivity. Indeed, much research has devoted attention to this fundamental question, as the size of the spine head is very tightly associated with the capacity of the synapse (Yuste, 2010). However, no one spine type appears to be more centrally desirable than other types. Indeed, increased numbers of thin spines in the nucleus accumbens are associated with clinical depression and may underscore many of the problems associated with conventional antidepressant treatment (Buyukdura et al., 2013). Despite the association between thin spines and capacity for plasticity, motivational deficits in the form of anhedonia persist in this condition. Thus, it would appear that the brain, under non-pathological functioning, maintains a diverse heterogeneity in spine population and morphology that produces appropriate functioning maximally suiting to meet the demands of the organism's environment. This line of reasoning is an extension of reflections proposed by Yuste (2010). Namely, dendritic spine populations maximize the distribution of connections within the brain and function in such a way to maximize computational power. As will be demonstrated in the coming discussion, deviations from normal spine populations can have very deleterious effects on overall function.

The main consequence of alterations of dendritic spine structure or number is aberrations in synaptic plasticity, namely the structural plasticity of the synaptic cleft (Herms & Dorostkar, 2016). During development through maturation, changes in spine density underlie synapse formation, maintenance, and elimination (Penzes et al., 2011).

Dendritic spines can additionally be altered in an experience-dependent manner. These changes can take place within a matter of minutes but can persist longer-term (Penzes et al., 2011; Herms & Dorostkar, 2016). The performance of certain tasks, such as motor tasks in animal models has been shown to promote an increased spine formation, for example. Indeed, in such cases, the formation of new dendritic spines has been shown to persist permanently throughout the life of the animal (Herms & Dorostkar, 2016). Extending this notion, rats and mice exposed to an enriched environment have shown significant effects of enrichment upon dendritic spine formation. These studies have further shown that enrichment not only increases the formation of dendritic spines, but also increases spine elimination, or turnover. The net result of this increase and subsequent turnover is an increased dendritic spine density, however, with significantly beneficial effects upon synaptic plasticity (Herms & Dorostkar, 2016). Consequently, cortical atrophy, as seen in many cases of neurodegenerative diseases, has significant effects on dendritic spine populations, with reductions in spine density and synaptic plasticity being key features of these neurophysiological conditions (Herms & Dorostkar, 2016).

CHAPTER 4

THE DISCOVERY OF DENDRITIC SPINES

While modern science has elucidated many of the processes associated with dendritic spines and synaptic plasticity, the story of dendritic spines is much older and dates to the mid-1800s. From their initial discovery, the processes associated with dendritic spines would not be made clear for nearly a century with the advent of more modern technology such as electron microscopy. The story of the dendritic spine is rife with conflict and disagreement. Even the initial discovery of the dendritic spine was labeled an artefact of the staining process that would eventually lead to their discovery. While the history of the dendritic spine is most clearly associated with Santiago Ramon y Cajal, the story of their discovery begins much earlier in Italy.

Camillo Golgi, the famous pathologist and eponymous discoverer of the Golgi apparatus, was born in the town of Corteno, a small town located close to Brescia in northern Italy (Droscher, 1998). Much of his scientific career is associated with the University of Pavia, located south of Milan, Italy. The genesis of Golgi's career came at a time of revolution within the Italian scientific community as a result of the Italian unification. Following initial revolutions in 1848, the constituent states located on the Italian peninsula moved toward unification and the formation of the 19th century Kingdom of Italy. This unification and the resulting surge of patriotism echoed through the halls of Italian academia, invigorating a new generation of scientists far removed from the isolationism and decadence of pre-unification Italian academia (Droscher,

1998). New experimental techniques and theories were imported from other countries and individual researchers were no longer stifled by the isolation imposed by the formerly individual Italian states (Droscher, 1998). Pavia, which was less isolated and separated from more contemporary science was perfectly situated to become a major influence in Italian biomedical research (Droscher, 1998).

One of the key figures to emerge from Pavia who plays a key role in the formative years of Camillo Golgi was the Italian pathologist Giulio Bizzozero, who introduced microscopy and cytology to Italian medicine (Droscher, 1998.) Bizzozero was born in Varese in 1846. He enrolled in the University of Pavia following his classical studies as a medical student under the tutelage of Paolo Mantegazza and later Rudolf Virchow (Droscher, 1998; Ribatti and Crivellato, 2007). Bizzozero graduated from medical school at the age of 20 and traveled abroad further studying pathology and microscopy under Rudolf Virchow and Heinrich Frey (Ribatti and Crivellato, 2007). Following his travel and education, Bizzozero established his laboratory and began to train one of the first generations of post-unification Italian pathologists in microscope techniques (Droscher, 1998). This training was invaluable to the developing career of Golgi, who stayed by Bizzozero's side as a friend until the latter's death from pneumonia in 1902.

Whereas Golgi's skill in microscopy was imparted by Bizzozero, his keen interest in neuroanatomy was imparted by the anthropologist and psychiatrist Cesare Lombroso. Driven by Darwin's theories, Lombroso was a proponent of evolutionary links between neuroanatomy and criminology (Mazzarello, 2011). His methods were not far removed from those of phrenology, with the beginning of his fascination with neuroanatomy coming from the examination of physical characteristics of the skulls of criminals

(Mazzarello, 2011). While Lombroso's work is now understood to be fraught with methodological issues, he is nonetheless regarded as one of the forefathers of criminal anthropology, with his fascination for neuroanatomy being imparted to the intellectually developing Golgi (Mazzarello, 2011).

Golgi's scientific interests were largely in the field of neurocytology, owing to the influences of Bizzozero and Lombroso. His technical mastery of microscopy, coupled with his insatiable fascination with neuroanatomy created a unique intellectual niche for Golgi. As a scientist, Golgi shared many characteristics with his contemporaries (Droscher, 1998). He was brilliant at the microscope and a consummate investigator of morphology and anatomy. However, he was not without shortcomings. Like many of his associates, he was very pragmatic but displayed difficulty in drawing conclusions and developing theories to form a conceptual framework for his considerable skill in cytology (Droscher, 1998). Though by all accounts a brilliant investigator, Golgi's career was not without struggle. Out of this struggle, however, emerged one of Golgi's most profound discoveries- a discovery which would later earn him international acclaim.

Following his graduation from medical school, Golgi was unable to locate a university position with adequate funding. He thus left the scientific community for a staff position at the *Pie case degli Incurabli*, in Abbiategrasso, not far from the University of Pavia (Droscher, 1998). Though far removed from the centers of inquiry and discovery that he had become accustomed to, he nevertheless continued his scientific career in his free time at his home. Golgi was compelled to develop a staining technique with greater resolution than what was available (Droscher, 1998). At that time, nervous system tissue was generally treated with potassium bichromate or osmium tetroxide as a

fixative and dyed with carmine. In the kitchen of his apartment, Golgi made the revolutionary discovery of treating tissue with silver nitrate. This process turned the neurons black and produced a resolution considerably greater than other techniques at the time. Owing to the black coloration of the neurons labeled with his new method, Golgi termed his reaction the *"reazione 38ear"*, or black reaction, and published his findings in 1873 in the *Gazetta Medica Italiani* under the title of *Sulla struttura della sostanza grigia del cervello*, which translates to "on the structure of the gray substance of the cerebrum" (Golgi, 1873; DeFelipe, 2015). This process of immersing the tissue in potassium or ammonium dichromate, followed by immersion in silver nitrate allowed for the constituent parts of neurons to be observed for the first time. Golgi's excitement at his discovery is reflected in a letter to his friend Niccolo Manfredi where he outlines his method (DeFelipe, 2015). His excitement was well placed, as his findings would later earn him a Nobel Prize in 1906.

This development marked a high point in Golgi's career. He was offered a professorship in histology at the University of Pavia and later earned the distinguished title of Professor of General Pathology (Droscher, 1998). Golgi refined his technique and began to investigate many regions of the nervous system including the olfactory bulb and Purkinje cell arborization in the cerebellar cortex. Previous staining methods allowed only for the visualization of the cell body and processes close to the soma. However, with the inception of Golgi's method, it was possible to follow the trajectories of individual processes and visualize more terminal regions of the cell (DeFelipe, 2015). This development marked a significant turning point in the history of neuroscience.

At the time of the development of Golgi's technique, the dominant theory among neuroscientists was that the constituent parts nervous system formed a continuum. This reticular theory, proposed by Joseph von Gerlach in 1872, dominated the field but was largely undermined with the inception of the Golgi procedure. Golgi examined tissue prepared by his own method and came to the conclusion that von Gerlach's reticular theory was indeed wrong (DeFelipe, 2015). However, Golgi's assertions were only partially correct. In contrast to the later neuron doctrine, Golgi proposed that the nervous system was a diffuse network, a finding that he termed "*rete nervosa diffusa".* As of the 1880s, Golgi's work had been translated furiously and he had received international recognition (Droscher, 1998). However, his proposal of the diffuse nerve network was met with considerable skepticism, particularly from the Spanish neurologist Santiago Ramon y Cajal, who will be presently discussed in great detail. Golgi's responsibility as a dean of the university left him very little time to respond to his denigrators, although he maintained his theory even after receiving the Nobel Prize (Droscher, 1998; DeFelipe, 2015). His theory of the "nerve net" likely comes from artefacts in the black reaction procedure. The partial blackening of tissue owing to the action of osmium appears very fine and net-like through a light microscope, which might explain the origin of Golgi's theory, though his *rete nervosa diffusa* is now universally regarded as being incorrect, owing largely to the research and tenacity of an incredible Spanish neuroscientist- Santiago Ramon y Cajal.

Santiago Ramon y Cajal was born in 1852 in the village of Petilla de Aragon, Spain. Born into a family with medical leanings, his father was a surgeon in the town of Petilla and later an anatomy teacher. His early years were perhaps not reflective of the

person who would later be a famous neuroanatomist with a Nobel Prize to his name (Taddeo, 2014). While his father tutored the young Cajal in geography, physics, and arithmetic, Cajal was somewhat absent-minded, preferring long treks throughout the Spanish countryside to socializing with other children (Taddeo, 2014). Cajal began to spend his days sketching his surroundings, much to the chagrin of his parents, who confiscated his artistic materials, wishing him to focus on a future medical career, much like his father (Taddeo, 2014).

In 1873, Cajal earned his medical degree from the University of Zaragosa in Spain and subsequently enlisted in the medical services of the Spanish army. The majority of his service was carried out during the Ten Years War, as Cuba wrestled with Spain for independence (Taddeo, 2014). Cajal's military career did not last long, however, as he was discharged following a diagnosis of malaria after only two years of service (Taddeo, 2014). His discharge from the military prompted Cajal to return to academic life, where he became a medical assistant at his alma mater. In 1878, Cajal earned a doctorate in anatomy from the University of Madrid, where he became well versed in microscopy and further honed his artistic talent (Taddeo, 2014).

Cajal's academic career ignited rapidly, as he became a professor of anatomy and later department chair at the University of Valencia, before transferring to the University of Barcelona, where he became professor and chair of histology (Taddeo, 2014). Perhaps the most crucial point in Cajal's academic life came following his discharge from the army during his doctoral studies in Zaragoza. It was at this time that he met Professor Aureliano Maestre de San Juan, who introduced Cajal to histological preparations (De Carlos & Pedraza, 2014). Cajal used his savings from his brief career in the military to

purchase a microscope, which he set up in his personal laboratory in his then home of Zaragoza, much like the previously discussed Camillo Golgi. In contrast to Golgi's extensive network of collaborators, however, Cajal preferred to work alone in his home laboratory, where he taught himself many histological techniques (De Carlos & Pedraza, 2014).

Though Cajal preferred the quiet peace of his home laboratory to the bustle of the University lab, he was not without collaboration and mentorship. One of Cajal's mentors who deserves mention is Luis Simarro Lacabra, who introduced Cajal to the Golgi staining method (DeFilepe, 2015). Lacabra was born in Rome and completed medical school in Valencia. Following an incident where Lacabra was discovered to be carrying out covert post-modern examinations in his capacity as director of the insane asylum, Sante Isabella at Leganes, he resigned his position and left for Paris where he attended anatomy lectures given by Louis Antoine Ranvier, one of the authors of *Manuel d'histologie pathologique* (Fernandez and Breathmach, 2001). Ranvier was an influential figure in French biomedical academia. Unlike the Italian and Spanish schools of histology, the French school remained skeptical of microscopic techniques. Ranvier was instrumental in establishing a place for microscopy in this school, as he, along with collaborator Edouard-Gerard Balbiani, established the first French journal devoted to microscopy, the *archives d'anatomie microscopique* (Appel, 1975; Fernandez and Breathmach, 2001). Ranvier, though a brilliant researcher, was described as unsociable and insensitive by his colleagues, and though admired, he was often feared by his students. His lectures remained unpopular owing to their technical detail and lack of theoretical concerns (Appel, 1975; Fernandez and Breathmach, 2001).

Despite Ranvier's arguable shortcomings as a teacher, Lacabra was nonetheless fascinated with Ranvier and learned a great deal from him, eventually exporting French histology techniques to his native Spain in 1875, two years after Golgi's landmark publication (Fernandez and Breathmach, 2001). Following his return to Spain, Lacabra continued his practice as a clinician out of necessity for financial support, though he held meetings of the Free Society of Histology in his private residence (Fernandez and Breathmach, 2001). Although it remains unclear whether he learned the Golgi method from Ranvier in Paris or from Golgi's 1873 manuscript, Lacabra was highly successful in preparing sections of the cerebral cortex using this method.

Though Golgi's method was indeed revolutionary, his success went unnoticed for a time, even being dismissed by Cajal himself, who did not consider it useful owing largely to dismissive writing employed by Ranvier, whose technical manuals significantly influenced Cajal's self-taught histology (DeFelipe, 2015). Cajal's attitude soon changed, however, when he visited Lacabra's home in Madrid in 1887. Lacabra, the ever-diligent student of histology, made significant modifications to Golgi's initial technique. Lacabra was well versed in photographic techniques owing to the mentorship of his uncle. Many of the skills he learned from photography were translated to the histological process in an attempt to improve the resolution of samples (Simarro, 1889; Fernandez and Breathmach, 2001). Cajal was immediately captivated, comparing the staining techniques to the fine sharpness of Chinese ink on rice paper (Cajal, 1909). It was this demonstration in which Cajal viewed what he considered to be the first good Golgi preparations and made him keenly aware of the importance of Golgi's work (Cajal, 1937; Fernandez and Breathmach, 2001). Cajal decided to return to his home in Valencia

armed with this new knowledge, excited for what he called a “brilliant future” of the technique (Cajal, 1937).

Independently, both Lacabra and Cajal began improving their procedures and maintained a friendship. Cajal’s improved formula included the introduction of hydroquinone and ammoniacal gold chloride, in addition to using a much higher concentration of potassium dichromate and a longer immersion time in silver nitrate (Cajal, 1909). Cajal’s influence from Lacabra and his fascination with the method led him to largely abandon general histology and focus more specifically on neurohistology (Cajal, 1937).

Cajal’s captivation with the method that he had learned quickly led him to use Golgi’s method to study and analyze neural tissue across a variety of species, often in the solitude of his home laboratory (DeFelipe, 2015). In just one year following his fateful meeting with Lacabra, Cajal had collected enough data to publish results obtained from his analysis of bird neural systems in his *Estructura de los centros nerviosos de las aves* (Cajal, 1888). Cajal (1888), made several conclusions that were important for the later development of the neuron doctrine. From his initial experimentation with Golgi’s method, Cajal began to notice several problems with von Gerlach’s reticular theory and Golgi’s diffuse network theory. In *Estructura de los centros nerviosos de las aves*, Cajal agreed with Golgi’s conclusion that dendritic spines do indeed end freely but argued that axons do as well, which was a significant departure from Golgi’s observations (Cajal, 1888; DeFelipe, 2015). Cajal acknowledged that while neuron fibers were interlaced in a complex manner, they never form a net and that each nervous cell is its on unit (Cajal, 1888).

Cajal's diligent efforts clearly paid off, as he shared the Nobel prize with Golgi in 1906 for his significant contributions to neuroanatomy and histology. Cajal did not rest on this laurel, however, and continued his research with the same fervent diligence that had characterized his earlier career. His subsequent research highlighted many examples from the nervous system of various species that confirmed his assertion that neurons end freely. While Golgi was known to have difficulty making conclusions and general theories about his work (Droscher, 1998), the same was not true of Cajal, who recognized the theoretical ramifications of the competing theories in explaining how current flowed through the nervous system (DeFelipe, 2015). The ideas of neuronal communication were widely different between Cajal's theories and the theories of Golgi and von Gerlach. Cajal's novel ideas about the composition of the nervous system led to novel theories about brain function. In his *Histologie du systeme nerveux de l'homme et des vertebres*, Cajal noted that while dendrites and axons end freely, the flow of neuronal information is not impeded or interrupted by the fragmented nervous system. He thus concluded that current must flow in a similar manner to how current travels along a splice between wires (Cajal, 1909).

Cajal's contributions to neuroscience were so profound that the so-called "neuron doctrine" was introduced and became the dominant theory in neuroanatomy by the end of the 19th century, even before Cajal's Nobel prize (DeFelipe, 2015). The term "neuron" was not introduced by Cajal, however, instead coming from an 1891 review by Wilhelm von Waldeyer-Hartz. This revolution in thought saw the neuron as the fundamental anatomical unit of organization within the nervous system. Cajal would go on to distinguish several types of connections between individual neurons, describing them as

"articulations" as early as 1897 (Cajal, 1933). These so-called articulations were later described as "synapses" by Foster and Sherrington (1897).

One of Cajal's most significant contributions, however, is the discovery and naming of the dendritic spine. Cajal noted that the surface of Purkinje cells that he examined appeared to be covered in "thorns" or short spines. Following his initial observations, he again observed these "short spines with small swellings" in pyramidal cells (Cajal, 1890). He thus interpreted these spines as possible targets for axons but did not further describe his theory until 1892. Cajal (1892) described these dendritic spines as being important features for contact transmission between the cerebellar and cerebral cortices, between Purkinje cells and pyramidal cells. In 1893, he discovered spines and projections in the pyramidal cells of the hippocampus and proposed that these structures facilitated contact with mossy fibers of the dentate gyrus (Cajal, 1893).

While independent researchers began to confirm Cajal's observations of dendritic spines, other authors denied that dendritic spines were any more than artefacts in the staining technique. Golgi believed that dendritic spines were nothing more than the crystallization of silver chromate on dendritic branches-a byproduct of the staining process. Consequently, many of their early descriptions and drawings did not include any mention of dendritic spines. At the time, dendritic spine visualization was only possible with the Golgi or the Golgi-Cox method (Cox, 1891; DeFelipe, 2015). Other methods such as the methylene blue technique were unable to label dendritic spines, thus dendritic branches appeared smooth when visualized with these methods, despite being comparable to the Golgi method in terms of image resolution (Meyer, 1895). While Cajal maintained the existence of dendritic spines from the consistency of the Golgi and Golgi-

Cox methods, he was not without doubt, as many authors at the time agreed with Golgi. Perhaps one of the greatest weaknesses in Cajal's self-taught methods was his admiration for the work of his contemporizes. Much like Cajal had initially dismissed the Golgi method from the writings of Ranvier, the work of Kolliker (1896) prompted Cajal to further investigate his position. Kolliker agreed with Golgi that dendritic spines were no more than artefacts of the staining process, and his *Hanbuch der Gewebelehre des Menschen* was studied and admired by Cajal, who was now determined to further investigate the existence of dendritic spines with histological techniques other than the Golgi method.

Cajal employed the methylene blue technique in an attempt to replicate the work of Meyer (1895). While Cajal found that this technique replicated earlier results of Meyer, he concluded that the dendrites were stained in such a way that spines were not visible under the microscope. Cajal thus modified the procedure and found that it was indeed possible to see dendritic spines with this method if the proper modifications were made (Cajal, 1896). Several other authors went on to confirm the existence of dendritic spines using this procedure as well (DeFelipe, 2015). Even Golgi himself later went on to publish an article in which he drew dendritic spines, however, the structures remain undescribed by Golgi in the manuscript as he did not attribute any particular function to dendritic spines (Golgi, 1901; DeFelipe, 2015).

While scientists gradually began to recognize the existence of dendritic spines, there was significant debate in the field as to their function. In particular, Held (1929), argued that dendritic spines were the end feet of axon terminals that fused with dendrites. He further proposed the existence of an interstitial network of nerves that allowed for

cellular communication due to anastomoses between dendritic spines and nerve fibers (Held, 1929). Cajal addressed this in a 1933 publication arguing that he had not seen such anastomoses between spines and nerve fibers, despite his long history of studying dendritic spines (Cajal, 1933). Cajal's publications included vast schematic drawings illustrating his argument. Cajal hypothesized that dendritic spines establish connections with axon terminals. In Cajal's later work, he described these connections as "synapses" borrowing from the earlier language of Foster and Sherrington (Foster and Sherrington, 1897; Cajal, 1933). While Cajal was indeed correct in his assertion, his revolutionary ideas would later be confirmed with the development and advent of electron microscopy.

Electron microscopy, while revolutionary for many reasons, allowed for the confirmation of many of Cajal's theories. Indeed, many of the first studies of dendritic spines involving electron microscopy heavily cite the work of Cajal as background (Gray, 1959). The first electron microscopy study confirming that dendritic spines were indeed structures essential for establishing synaptic connections comes from Gray (1959). Gray (1959) replicated previous tissue preparations intended for analysis with light microscopy with electron microscopy and found that while the pre-synaptic end-feet were only just visible with light microscopy, they were clearly visible with the more advanced electron microscopy. This work was replicated by Gray and Guillery, (1966), although the functional significance of dendritic spines would remain somewhat unclear (Scheibel and Scheibel, 1968).

Jones and Powell (1969) provided one of the first large examinations of morphological variations in dendritic spines in the neocortex of felines in a sample of 25 feline brains. They characterized and described the morphology of the observed dendritic

spines in great detail. Particularly, they noted the ease of distinction of dendritic spines from other cellular components by the absence of typical dendritic inclusions, neurotubules, ribosomes, and neurofilimants (Jones and Powell, 1969). Moreover, this manuscript provided one of the first concise morphological descriptions of dendritic spines, showing that spines can vary greatly in shape, size, and in attachment to their parent dendrites (Jones and Powell, 1969). Furthermore, this manuscript provided one of the first major morphological distinctions between types of spines, distinguishing spines as either rounded, cup-like, or prismatic (Jones and Powell, 1969). Particularly relevant to the concern surrounding dendritic spine function discussed by Scheibel and Scheibel, (1968), Jones and Powell observed that each dendritic spine receives an axon terminal that contains synaptic vesicles, concluding that these synapses should be excitatory in nature (Jones and Powell, 1969). The authors concluded their discussion by suggesting that the functional role of dendritic spines is much more complicated than the prevailing notion that dendritic spines only served to increase the surface area of axonal contacts, due to the great variation in spine shape, size, and location. While future research was indeed necessary to fully flesh out the function of dendritic spines, Jones and Powell (1969), presented arguments that were incredibly insightful for their time. First, there are indeed morphological differences in dendritic spines that are directly related to their functions (Yuste, 2010). Secondly, as hypothesized, the majority of dendritic spines do indeed establish at least one excitatory synapse, with only a very small percentage of dendritic spines found to be non-synaptic (Yuste et al., 2010; DeFelipe, 2015).

While the observations of Gray (1959) would be replicated by numerous authors, more direct demonstrations showing that dendritic spines establish synaptic connections

came from the work of Fairen and colleagues (1977). Fairen et al., (1977) employed de-impregnated Golgi stained neurons to study finer cytoarchitectural details which were previously unable to be examined using more traditional Golgi methods. Extracted rat brains were processed by perfusion of formaldehyde and the pieces were rapidly stained using Golgi staining techniques. Cut brain sections were then immersed in gold chloride and oxaclic acid before removal of silver chromate impregnation by sodium thiosulphate. While the silver chromate was removed from the tissue, the treatment of gold chloride left behind a fine trace of gold in the previously Golgi-stained neurons. When viewed under an electron microscope, these fine deposits of gold were readily visible and marked the profiles of the previously stained Golgi neurons, while leaving the fine structural details clearly visible (Fairen et al., 1977). Processes of the neuron which were previously not visible using conventional Golgi methodology were now clearly visible due to the impregnation of the tissue with gold particles. These fine gold tracing allowed the ultrastucture and synaptic connectivity of the neurons to be clearly distinguished when viewed through an electron microscope (Fairen et al., 1977; DeFelipe, 2015).

The additional development of advanced methodology in immunohistochemistry soon allowed for an examination of the connectivity and characteristics of neurons labeled with the Golgi method. Frotscher and Leranth (1986) combined choline acetyltransferase immunohistochemistry with Golgi staining methods to characterize the connectivity of granule cells in the fascia dentata of the rat. This study also provided one of the first correlational studies between light and electron microscopy (Frotscher and Leranth, 1986; DeFelipe, 2015). Choline acetyltransferase immunoreactive axons were examined first with light microscopy, where a fine varicosity of axons innervating the

fascia dentata was observed running parallel to the granule cell layer. (Frotscher and Leranth, 1986). Immunostained sections were then covered in agar and stained using Golgi methods. Following this staining, sections containing well-labeled cells were toned with gold in a procedure similar to Fairen et al (1977). Axon terminals that were immunoreactive to choline acetyltransferase were shown to establish synaptic contacts with the cell bodies and dendrites of the gold impregnated granule cells. These results characterized the cholinergic system in the fascia dentata and established the synaptic connectivity of neurons in this region and was one of the first methods to combine electron microscopy, Golgi impregnation, and immunohistochemical staining methods (Frotscher and Leranth, 1986; DeFelipe, 2015). These initial examinations paved the way for examinations of the details of efferent and afferent connections and a further examination of the role of dendritic spines in synaptic communication (DeFelipe et al., 2015). In the years following these findings, dendritic spines have consistently been demonstrated to play key roles in learning, memory, cognition, and affect (Yuste, 2010).

The history of the discovery of dendritic spines is a fascinating story of scientific discovery, innovation, argument, and achievement. From the development of novel techniques by Camillo Golgi to the persistent observations of Cajal, this history underpins much of modern neuroscience. The discovery of the dendritic spine represents the combined efforts of scientists representing Italian, Spanish, French, and German academic traditions of cytology, microscopy, neuroscience, and histology. Camillo Golgi's revolutionary technique allowed for the first visualizations of the dendritic spine, though they would be dismissed as simple artefacts of the staining process. Santiago Ramon y Cajal, through his interaction with Luis Simarro Lacabra and by proxy

interaction with Louis Antoine Ranvier, would go on to consistently improve the Golgi staining procedure and repeatedly argue for the existence of dendritic spines. Though his efforts were not without detractors, Cajal's single-minded persistence in the laboratory and his insistence on both the neuron doctrine and the existence of dendritic spines would pave the way for generations of neuroscientists to investigate one of the most remarkable features of neuronal anatomy and physiology. The confirmation of many of Cajal's ideas by the application of electron microscopy gave rise to more modern understandings of neuroanatomy, neurophysiology, and synaptic plasticity. From humble beginnings as mere artefacts of the Golgi procedure, dendritic spines are now understood to critical anatomical features underlying much of neurotransmission. Additional findings have further demonstrated their involvement in a wide array of behavioral and cognitive functions.

CHAPTER 5

HIV, DEPRESSION, AND DENDRITIC SPINES

Dendritic injury has been thought to be an underlying mechanism of HIV-associated neurocognitive disorders (Ellis et al., 2007; Moore et al., 2006). Indeed, Ellis et al., (2007) argue that antiretroviral medication supplemented with neuroprotective agents might be therapeutically beneficial in promoting plastic and improved clinical status of patients suffering from forms of HAND. This relationship between neuropathology and HAND is further reflected in Moore et al. (2006), which examined postmortem brain tissue correlates with antemortem clinical data and found significant correlations between impairment and damage, particularly with respect to the hippocampus and putamen (Moore et al., 2006).

Synaptic degeneration is arguably one of the most severe neuropathologies associated with HIV and likely contributes to the high rates (50% of seropositive patients) of HIV-associated neurocognitive disorder, with neurocognitive impairment being a direct result of synaptic degeneration (Everall et al., 1999; Bellizzi et al., 2006; Ellis et al., 2009). Following HIV infection during seroconversion, roughly 50% of HIV-infected individuals experience acute HIV syndrome which may consist of meningitis, myelopathy, or encephalopathy (Ru & Tang, 2017). Following seroconversion, white matter aberrations are noted, although there is typically not an associated loss of neurons during the asymptomatic period of HIV infection. Vast neuropathological changes are associated with the progression to AIDS, often including HIV-associated encephalitis.

Neuronal apoptosis is typically associated with this stage, although non-apoptotic injuries are common as well (Ru & Tang, 2017). Though HIV-associated encephalitis and neuronal apoptosis are associated with severe dementia, they do not appear to be significantly correlated with milder forms of cognitive impairment (Adle-Biassette et al., 1999; Ru & Tang, 2017). Non-apoptotic synaptic injury, on the other hand, is closely associated with the presence and severity of HIV-associated neurocognitive disorders. HIV-mediated synapse loss in the early stages of HAND precedes neuronal apoptosis, with surviving neurons demonstrating a remarkable capacity for synaptic repair if given the proper treatment (Bellizzi et al., 2006; Kim et al., 2008).

HIV-mediated synaptic loss appears to be closely associated with the inflammatory environment produced by HIV viral proteins such as tat and gp120. Gp120 released by astrocytes has been shown to produce dendritic vacuolization, spine loss, and loss of presynaptic terminals (Toggas et al., 1994). Synaptic damage that proceeds by way of Tat is a distinct process from full neuronal death (Kim et al., 2008). Cellular death in the presence of HIV is mediated by low-density lipoprotein receptor-related protein. Activation of LRP results in subsequent activation of the NMDA receptor producing a calcium-mediated rise in nitric oxide synthase leading to cell death (Kim et al., 2008). Synaptic damage, on the other hand, is a result of Tat produced, proteasome-mediated degradation of micro-tubule-associated protein 2 (MAP2), which consequentially results in a collapse of cytoskeletal filaments (Kim et al., 2008). Although synaptic damage is produced by a similar pathway that cellular death is, there is an important bifurcation in the pathway that suggests that synaptic damage may be a compensatory mechanism (Kim et al., 2008; Green et al., 2019).

Whereas cellular death requires calcium-mediated neuronal nitric oxide synthesis, synaptic damage associated with tat is not mediated by nNOS, but rather ubiquitin-proteasomal pathways (Aprea et al., 2006; Kim et al., 2008). Tat-induced synaptic damage has been shown to accompany learning and memory deficits in addition to flexible attention deficits in rodent models (Fitting et al., 2012.) Moreover, the cysteine-rich region of the tat protein has been shown to play a critical role in the early development of synaptic degeneration (Bertrand et al., 2013).

More recently, HIV- mediated synaptic changes have been suggested to result from a homeostatic scaling response. Indeed, HIV-induced synaptic loss may result from a compensatory process to avoid cellular death (Green et al., 2019). HIV viral proteins and inflammatory cytokines in the brain result in excessive activation of glutamatergic receptors. Thus, the "scaling down" of synaptic connections may indeed be a neuroprotective mechanism that protects from cellular damage and excitotoxicity, rather than a symptom of the beginning stages of neuronal apoptosis (Green et al., 2019). Such an explanation would additionally explain the enormous temporal gap between synapse loss and neuronal death typically observed within HIV seropositive patients (Green et al., 2019). Such scaling is reversible, however, and may potentially serve as a therapeutic avenue (Green et al., 2019).

The idea that HIV-mediated synaptic damage is a consequence of homeostatic scaling is far from perfect however. Much of the evidence used to support the ideas indicated in Green et al., (2019) appears to be derived from *in-vitro* investigations. Few, if any studies, have addressed this hypothesis *in vivo,* with fewer investigations targeted

toward clinical demographics. Moreover, the notion that HIV-induced synaptic damage is a compensatory mechanism hinges largely on the finding that synapse loss in HIV-1 is mediated by NMDA receptor activation. Specifically, HIV viral proteins and cytokines produce a rapid potentiation of NMDA activity via kinase signaling cascades including Src pathways, in addition to protein kinase A and C pathways (Green et al., 2019). If indeed synaptic scaling in HIV-1 occurs as a neuroprotective mechanism to avoid excessive glutamatergic activation, NMDA antagonists would likely show promise in reversing HIV-1 mediated synaptic damage. However, few NMDA receptor antagonists have been shown to be an effective treatment for synapse loss in HIV (McGuire et al., 2014). NMDA antagonists (such as memantine) have been investigated as a potential adjuvant therapy to cART but do not appear to have any dramatic effect upon the manifestation or intensity of HAND in clinical populations (McGuire et al., 2014). Similarly, GABAergic-based interventions such as valproic acid have likewise failed to produce alterations in HAND (McGuire et al., 2014). Other NMDA non-competitive receptor antagonists such as Dizocilpine have shown to inhibit Tat-induced synapse loss, though the role of NMDA receptor antagonists in the treatment of HIV remains somewhat unclear (Shin et al., 2012). Collectively, while it would appear that HIV-1 induced spine damage is a consequence at least in part of excessive NMDA activation, the exact mechanisms behind HIV associated spine loss remain elusive under this synaptic scaling account.

HIV-induced synaptic loss may result from a compensatory process to avoid cellular death (Green et al., 2019), thereby altering circuit connectivity (Illenberger et al., 2020). HIV viral proteins and inflammatory cytokines in the brain result in excessive

activation of glutamatergic pathways, particularly those frontostriatal pathways which are critically involved in apathy. Previous reports highlight the reversibility of HIV-1 induced dendritic damage (Bellizzi et al., 2006; Kim et al., 2008; Kim et al., 2011; Bertrand et al., 2014; Denton et al., 2020), but more research is needed to more fully elucidate effective treatments for HIV-1 induced synaptodendritic damage, though phytoestrogen treatment, cannabinoid receptor activation antidepressant treatment are promising treatment avenues (Kim et al., 2011; Bertrand et al., 2014; Denton et al., 2020). Full restoration of synaptodendritic function may be problematic, however, as if synaptic damage is indeed a compensatory mechanism that protects against excitotoxicity, restoration of the synaptic network may have the unintended consequence of apoptosis (Kim et al., 2008; Green et al., 2019). This caution has led some researchers to surmise that effective treatments for HIV-induced synaptodendritic damage should be neuroprotective in nature and ideally act upstream of the bifurcation in the pathway that leads to apoptosis or synaptodendritic damage, which was described above (Kim et al., 2008). Moreover, it has been suggested that an effective strategy to improve synaptodendritic connectivity in HIV would be to introduce therapeutics at targets that are completely separate from mechanisms that improve cellular survival (Bellizzi et al., 2006; Kim et al., 2008). Additional therapeutic attempts should consider these suggestions and ensure that synaptodendritic repair does not occur at the expense of excitotoxicity.

The HIV-1 Tg rat provides invaluable information concerning the relationship between dendritic spine dynamics, dopaminergic function, and HIV-1 infection. Roscoe et al., (2014) examined the effects of HIV-1 infection upon spine population dynamics in

the nucleus accumbens of HIV-1 Tg rats and discovered several interesting findings that are still presently discussed and deliberated within the literature, with attenuation of spine shift dynamics presenting a potential therapeutic target for intervention in cases in HIV-1 (McLaurin et al., 2018; Denton et al., 2020). Indeed, shifts in spine dynamics may play a central role in underlying the well-documented dopaminergic pathology associated with HIV-1 infection (Denton et al., 2019), although potential explanatory mechanisms remain unclear (Denton et al., 2020).

Roscoe et al., (2014) was the first published attempt to examine dendritic spine populations in the nucleus accumbens of HIV-1 Tg rats. Medium spiny neurons (MSNs) comprise a major inhibitory projection in the nucleus accumbens and are hypothesized to play a major role in motivation and reward owing largely to the dopaminergic innervation in the area. Particularly relevant to the present discussion of HIV-1 and apathy, dysfunction of the nucleus accumbens is associated with both apathy and depression (Nestler and Carlezon, 2006). The nucleus accumbens receives dopaminergic input from the ventral tegmental area in the midbrain and is postulated to serve a major role in reward both with respect to addiction and natural rewards such as food and social interaction (Nestler and Carlezon, 2006). Naturally, dysfunction related to the nucleus accumbens is a prime candidate for a mechanism underlying disruptions in reward such as apathy and anhedonia and is of particular relevance to discussions of HIV and apathy. Shifts in dendritic spine distributions and dendritic proliferation often are indicative of underlying neuropathologies (Roscoe et al., 2014). Decreased dendritic branching was immediately obvious in the HIV-1 Tg rat. Control animals displayed a more complex branching pattern, with multiple proximal processes extending from the cell body and a

full arborization pattern (Roscoe et al., 2014). In contrast, HIV-1 Tg rats displayed significantly blunted arborization patterns, with significant shifts in spine population as a function of transgene. Both spine length and spine volume were significantly altered in HIV-1 Tg animals, although head diameter remained unchanged between control and HIV-1 Tg rats.

Roscoe et al., (2014), demonstrated that chronic HIV-1 protein exposure (as is the case with the HIV-1 Tg rat model) potentially leads to synaptodendritic injury in the nucleus accumbens. This finding is particularly in line with the present discussion of dendritic spines and dopaminergic functioning. Medium spiny neurons play a major role in motivational and reward systems. Damage to these systems and consequent disruption in motivation and reward is in line with clinical definitions of anhedonia and apathy. Indeed, Roscoe et al., (2014) reported that HIV-1 Tg rats demonstrated a population shift in dendritic spines from longer spines with defined heads to shorter, stubbier spines. This finding highlights the altered synaptic connectivity in the HIV-1 Tg rat, as shorter spines have compromised synaptic connectivity (Roscoe et al., 2014). Thus, this shift in dendritic spine populations may be a potential explanatory mechanism underlying findings of impaired dopaminergic transmission in the HIV-1 Tg rat, although as will be shown shortly, the relationship may be more complex than a one to one mapping of structure to function.

Further extending the findings of comprised synaptodendritic integrity in the HIV-1 Tg rat, McLaurin et al., (2018) again found an alteration in dendritic spine distribution using a similar procedure to what was previously described in Roscoe et al., (2014). Additionally, a Sholl analysis was employed to quantify neuronal arborization.

Profound decreases in arborization of medium spiny neurons in the nucleus accumbens were reported, with biological sex also a significant mediatory factor (McLaurin et al., 2018). These findings further illustrate the role of synaptic complexity underlying the neuronal pathology in HIV-1. As previously discussed, the nucleus accumbens receives major dopaminergic input from the VTA and plays a large role in motivation and reward. In HIV-1 Tg animals, the presence of stubby spines at dendrites farther from the soma might be indicative of decreased innervation from the VTA (McLaurin et al., 2018). A distinct morphological characteristic of stubby spines is the absence of a dendritic spine neck. This absence underlies deficits in neural communication that might suggest a profound decrease or even a lack of dopaminergic innervation in the nucleus accumbens from the ventral tegmental area (McLaurin et al., 2018). Synaptic alteration again suggests a neuroanatomical mechanism underlying dopaminergic deficits in the HIV-1, and, by extension, apathy. Moreover, findings from studies examining the prefrontal cortex have demonstrated profoundly compromised synaptodendritic integrity (McLaurin et al., 2019). Specifically, pyramidal neurons in layers II and III of the prefrontal cortex display significant alterations in branching complexity and spine morphology (McLaurin et al., 2019). Given the relationship between the prefrontal cortex and reward pathways in the brain, it is likely that severely compromised synaptodendrtic connectivity in these regions underly both HIV associated apathy and depression, as previous reports have indicated compromised serotonergic dysfunction in the prefrontal cortex as well (Denton et al., 2019). Additionally, medium spiny neuron dysfunction has been demonstrated to be associated with depression (Francis et al., 2015).

Similar to the findings reported in Roscoe et al., (2014) and McLaurin et al., (2018), Denton et al., (2020) reported a profound shift in dendritic spine populations in the nucleus accumbens of the HIV-1 Tg rat. As reflected in the previous discussion, spine pathologies in the HIV-1 Tg rat are hypothesized to provide a potential explanatory basis for the occurrence of conditions such as apathy and anhedonia (Bertrand et al., 2018; Denton et al., 2021). Denton et al., (2021) investigated the effects of SSRI treatment upon behavioral, electrochemical, and neuroanatomical markers in the HIV-1 Tg rat. Similar to results presented by Roscoe et al., 2014 and McLaurin et al., (2018), significant shifts in dendritic spine populations were reported. HIV-1 Tg animals demonstrated profoundly reduced dendritic complexity, as analyzed by a Sholl analysis. Moreover, HIV-1 Tg animals demonstrated shifts across differentiated spine types (Denton et al., 2021.)

The mechanism(s) by which significant spine changes occur in depression is not well understood. Several synapse-related genes have been implicated in a number of studies, but a conclusive target remains to be elucidated (Qiao et al., 2016). Moreover, depression and stress-induced disruption of chemical signaling pathways such as cAMP have been hypothesized and examined (Qiao et al., 2016). Of the more promising targets underlying synaptic shifts in depression is the disruption of mTOR signaling (Qiao et al., 2016). However, any one mechanism is unlikely to account for the profound changes observed. Shifts in dendritic spine populations in depression are largely region-dependent. Certain areas of the brain have been shown to display decreased spine numbers while other areas have been shown to display *increased* spine numbers. The reasons for discord among these areas remain unclear, and there does not appear to be a promising hypothesis that could account for the variety of changes in these areas.

Regions such as the hippocampus and prefrontal cortex consistently show decreases in spine density in animal models of depression such as the chronic social defeat paradigm, chronic unpredictable stress paradigm, and chronic restraint stress paradigms. However, dopaminergic areas, in particular the nucleus accumbens, show an *increase* in spine density. This variety of disparate findings serves to illustrate the complexity of the neurochemical changes associated with depression.

With respect to stress paradigms of depression, dendrites of pyramidal cells in the CA3 region of the hippocampus show significantly greater atrophy than areas such as the CA1, likely owing to differential sensitivity as a function of serotoninergic and GABAergic receptors (Qiao et al., 2016). Dendritic damage associated with chronic stress in the CA3 region is also reversible if a period of non-stress is introduced into experimental paradigms (Qiao et al., 2016). Stress-induced changes in the CA1 are far less characterized, and mediated by biological sex (Qiao et al., 2016). Fluctuations of spine density in this region are likely dependent upon NMDA receptor activation (Qiao et al., 2016). Spine density changes in the prefrontal cortex follow similar patterns as those displayed in the hippocampus. Animal models of depression typically reduce dendritic proliferation in pyramidal cells of layers II and III, accompanied by a profound decrease in spine density (Qiao et al., 2016). Similar to the hippocampus, spine changes in the prefrontal cortex are mediated by biological sex, but also age (Qiao et al., 2016). Similar decreases in spine density are observed in the orbitofrontal cortex, although the length of apical dendrites in this region has been observed to be extended by stress-based paradigms of depression (Qiao et al, 2016). The exact mechanisms by which comparable stressors could reduce dendrite length in one region but increase it in another remain a

source of speculation, although potential mechanisms such as Kalirin-7 expression have been proposed (Qiao et al., 2016). A completely opposite effect is observed in the amygdala, however, with increases in both spine density and arborization demonstrated in response to chronic stress models of depression, which mirrors findings from fear conditioning studies (Qiao et al., 2016).

Returning to our discussion of depression and dopamine, spine changes in the nucleus accumbens are opposite from those recorded in the prefrontal cortex, with increases in spine density reported in response to chronic stress models of depression (Qiao et al., 2016). Paradoxically, findings from clinical patients show a significantly reduced overall volume of the nucleus accumbens. Excitatory glutamatergic axons synapse onto medium spiny neurons in the nucleus accumbens, which receive dopaminergic input from the ventral tegmental area. The ventral tegmental area consequently receives GABAergic input from the nucleus accumbens and glutamatergic inputs from the prefrontal cortex. This functional regulation of medium spiny neuron population activity is highly disrupted in depression (Qiao et al., 2016).

Findings surrounding spine populations and dopaminergic transmission have been mixed. Medium spiny neurons compose 95% of the dorsal striatum and nucleus accumbens. Of this neuron population, roughly half of striatal medium spiny neurons express predominantly D1 receptor subtypes, while the other half expresses D2 receptor subtypes. D1 and D2 colocalization in this region is estimated to be no greater than 30% (Bertran-Gonzalez et al., 2008; Qiao et al., 2016). D1 signaling has been shown to enhance excitation and glutamatergic signaling, while D2 signaling in this region has demonstrated the opposite effect (Smith et al., 2013; Qiao et al., 2016). One such finding

is that medium spiny neurons expressing predominantly D1 receptors exhibit reduced dopaminergic activity in established animal models of depression (Francis et al., 2015; Qiao et al., 2016). Enhanced activity of these neurons is associated with recovery, while decreased activity is associated with depression (Francis et al., 2015; Qiao et al., 2016). Additional findings suggest that chronic stress models of depression in mice result in an increase of synaptic strength measured by postsynaptic currents in mushroom spines on D1 medium spiny neurons in stress-resilient mice (Khibnik et al., 2015; Qiao et al., 2016). This increase in synaptic strength is accompanied by a decrease in strength of predominantly D2 receptor containing medium spiny neurons. In non-resilient mice, the postsynaptic current amplitude of both mushroom and thin spines is not affected on D1 or D2 receptor containing medium spiny neurons (Khibnik et al., 2015; Qiao et al., 2016). KappaB kinase in the nucleus accumbens has been shown to play a role in the development of anhedonic behavior, while kappaB nucleus factor regulates synaptic morphology (Qiao et al., 2016). Overexpression of kappaB kinase is associated with an increase in thin spine density in the medium spiny neurons and promotes social withdrawal in rodents (Christoffel et al., 2012; Qiao et al., 2016). Similarly, increased levels of kappaB kinase in the nucleus accumbens are correlated with increased stubby spine density, which are both highly correlated with depressive phenotypes (Christoffel et al., 2011; Qiao et al., 2016). Collectively, these findings illustrate that KappaB kinase and its associated factors regulate depressive behavior and synaptic morphology in the nucleus accumbens mainly through an increase in spine density of thin and stubby spine types. Thus, increases in spine density in the nucleus accumbens associated with depression are largely mediated by thin and stubby spine subtypes.

As one can hopefully understand from the discussion up until this point, HIV and depression have consider shared features in terms of synaptic reorganization and consequent neurochemical impairment. Even in the absence of comorbidities, depression and HIV are both characterized by synaptic rewiring of the brain to a more juvenile state. This rewiring of the brain likely has deleterious consequences in terms of synaptic transmission. Indeed, HIV has been shown consistently to involve significant dopaminergic and serotonergic impairments (Denton et al., 2019; Denton et al., 2021) which have likewise been shown to accompany clinical depression (Nestler and Carlzzon, 2006).

CHAPTER 6

TELOMERES, NEUROGENESIS, AND DOUBLECORTIN

Telomeres are repeated sequences of 5'-TTAGGG-3' sequences present at the end of chromosomes that protect from chromosomal degradation and recombination (Zhang et al., 2016; Vakonaki et al., 2018). Telomere shortening is associated with the normal aging process. While aging is biologically natural, the gradual loss of cellular function and integrity will eventually result in the functional decline of organs and tissue (Zhang et al., 2016). This gradual decline eventually results in common age-related diseases. Conditions such as dementia, cancer, and atherosclerosis are common conditions associated with natural aging processes and the subsequent reductions in tissue integrity (Singh and Newman, 2011). In this way, telomeres serve as the body's natural cellular clock, with rates of telomere shorting directly impacting the aging process (Zhang et al., 2016). Among the cellular and molecular processes that underlie the aging process, telomere attrition and chronic inflammation are considered to be two of the most common underpinnings (Zhang et al., 2016).

Gradual telomere attrition is a hallmark of the aging process and is a natural occurrence throughout the lifespan of organisms. However, risk factors such as inflammation, oxidative stress, and even genetic factors have the potential to accelerate telomere attrition (Aubert and Lansdorp, 2008; Zhang et al., 2016). Moreover, telomere attrition is associated with an increase in cellular senescence, or the stable and long-term loss of cellular proliferation capacity (Zhang et al., 2016). While senescent cells are still

metabolically active, they possess an altered secretory phenotype which is likewise associated with an increase in the production of proinflammatory cytokines (Zhang et al., 2016). This process of cellular senescence likely contributions to the chronic inflammation that is connected with cellular aging, as inflammatory mediators are constantly up-regulated during the aging process (Rodier and Campisi, 2011). Thus, the limited replicative capacity of cells is directly contingent upon telomere shortening, a phenomenon known as the Hayflick limit (Hayflick and Moorhead, 1961; Zhang et al., 2016).

Overall telomere length is the net outcome of lengthening mechanisms such as telomerase and the alternative lengthening of telomere (ALT) mechanism as opposed to shortening mechanisms such as active end processing and replication (Zhang et al., 2016). Telomere function depends critically on the stability of the telomeric nucleoprotein structure and its length, as a minimum number of telomeric repeats is required to allow shelterin complex protein binding (Bendix and Kolvraa, 2010). Shelterin proteins mediate the formation of telomere structures during replication and prevent chromosomal ends from being mistaken as DNA breaks during the natural replication process (Wu et al., 2012). The shelterin protein complex consists of six proteins: telomeric repeat-binding factor (TRF) 1 and 2, TRF-1-interacting protein 2, repressor-activator protein, TPPI, and protection of telomeres protein (Palm and de Lange, 2008). Collectively, these proteins ensure correct telomere formation and prevent end-to-end chromosomal fusions (Palm and de Lange, 2008).

Increases in rates of inflammation are associated with increases in cellular replication and consequent telomere attrition (Zhang et al., 2016). These increases in

telomere attrition and cellular replication expedite cellular senescence and the consequent aging process (Zhang et al., 2016). Moreover, telomere shorting is commonly accelerated due to oxidative stress, which is a common association with inflammation. The G-rich telomere sequences are particularly vulnerable to oxidative stress. Oxidative stress results in the formation of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG) formation at the GGG sequence of telomeres, which is likewise cleaved by formamidopyrimidine-DNA glycolyase, which has the direct consequence of excessive loss of telomere sequences (Kawanish and Oikawa, 2004). Moreover, oxidative stress may prevent the binding of the shelterin complex proteins, which further increases cellular senescence (Sun et al., 2015).

Telomere length shortening has been described as a characteristic of major depressive disorders (McEwen 2003; Hammen 2005; Alloy et al., 2010; Vakonaki et al., 2018) and HIV infection (Oeseburg et al., 2010; Auld et al., 2016), both of which critically involve inflammation and oxidative stress. Moreover, viral protein production has been shown to increase inflammation in the HIV-1 Tg rat (Royal et al., 2007; Homji et al., 2012; Royal et al., 2012; Vigorito et al., 2015). While these reports have somewhat elucidated the relationship between telomeres, inflammation, and conditions such as HIV and depression, the collective understanding of the field in this regard is still somewhat primitive, with many questions to be answered. Few studies, if any, have directly examined the joint effects of HIV comorbid depression on inflammation, oxidative stress, and telomere length. Moreover, with regards to depression, the exact relationship between telomere length, inflammation, and antidepressant treatment remains somewhat of a mystery, with too few reports being produced to garner an appropriate understanding of these phenomena.

Switching gears to a discussion of neurogenesis and doublecortin (DCX), DCX is a microtubule-associated phosphoprotein commonly utilized as a marker of newly formed neurons in the dentate gyrus and is consequently a biomarker of neurogenesis (Rao and Shetty, 2004, Ayanlaja et al., 2017). Hippocampal neurogenesis occurs as additional granule cells are added to the dentate gyrus throughout the lifespan of an organism (Hill et al., 2015) Increases in hippocampal neurogenesis have previously been reported as a feature of antidepressant adherence, and even as a requirement for behavioral effects of treatment (Malberg et al., 2000; David et al., 2009; Boldrini et al., 2009; Hill et al., 2015).

Microtubules play a critical role in cell motility, shape transport, and polarity, and are regulated by microtubule-associated proteins (Ayanlaja et al., 2017). These proteins influence microtubule stabilization and organize cellular and cytoskeletal components (Ayanlaja et al., 2017). DCX is 40 kDA microtubule-associated phosphoprotein that is encoded by the DCX gene located on the X chromosome. This protein is specific to the nervous system and plays a critical role in cell migration during the early development of the nervous system (Ayanlaja et al., 2017). Mutations in the DCX protein cause abnormal neuronal migration which results in a defective organization of the cortex (Ayanlaja et al., 2017). Proper migration during the early stages of neuronal development is essential to the development and function of the nervous system. Complete neuronal function is contingent upon a series of cytoskeletal modifications that include adjustments to cell membranes, adhesive structures, and the interaction with proteins. These modifications are largely a result of the actions of microtubules and microtubule-associated proteins, of which DCX is one of the most critical (Ayanlaja et al., 2017).

DCX has been shown to play an important role in the proliferation of progenitor cells during neurogenesis (Ayanlaja et al., 2017). Moreover, it is essential for neuronal differentiation and cell migration as a result of its involvement in the stabilization of microtubules (Ayanlaja et al., 2017). Owing to these features, DCX has been adopted as a biomarker of neuron precursors and migrating neuroblasts during adult neurogenesis (Brown et al., 2003). Adult-born neurons have been examined in sheep, rats, mice, voles, and hamsters, which suggests that hypothalamic neurogenesis is a conserved process across many mammal species (Ayanlaja et al., 2017).

Adult hippocampal neurogenesis is the process by which additional granule cells are gradually added to the dentate gyrus throughout the lifespan of the organism. These new neurons are produced from progenitor cells located in the subgranular zone of the dentate gyrus (Hill et al., 2015). Many environmental factors can impact the rates of proliferation, maturation, and survival of these newly formed neurons. Factors such as age, stress, and exercise have been naturally shown to impact proliferation and turnover of adult-born neurons in the hippocampus (Hill et al., 2015). Moreover, antidepressant adherence has been shown to alter neurogenesis in the hippocampus (Hill et al., 2015). The relationship between hippocampal neurogenesis and antidepressant adherence is not completely clear, however. While antidepressant medication has been promoted and prescribed for many decades, the full scope of the mechanisms of action for antidepressant medications remains somewhat elusive. Most commonly prescribed antidepressants act through direct interaction with the monoamine neurotransmitters in the brain. While antidepressants have the capacity to change the direct concentrations of monoamine levels within a matter of hours, direct behavioral effects and changes in

mood are typically not observed for a period of weeks to months following the onset of treatment (Hill et al., 2015).

An early hypothesis concerning the relationship between antidepressants and behavior was posited after the observation that antidepressant-induced neurogenesis occurs roughly over the same time course that is required to observe the behavioral effects of antidepressants. These initial observations allowed researchers to suggest that the behavioral effects of antidepressant treatment may be a direct consequence of hippocampal neurogenesis (Duman et al., 2001; Toni et al., 2007; Hill et al., 2015). Since the initial onset of these observations, hippocampal neurogenesis has been demonstrated to be required for some behavioral effects of antidepressant treatment, but not all of the observed effects (David et al., 2009).

In a 2015 review, Fields et al. highlight evidence showing that the HIV-1 viral protein Tat promotes calpain-1 cleavage of p35 to p25 via a mechanism of calcium dysregulation. This produces a hyperactivation of CDK5 producing abnormal phosphorylation of downstream DCX (Fields et al., 2015). Alterations in hippocampal neurogenesis have been noted in the brains of HIV seropositive individuals, and are potentially linked to HIV-associated neurocognitive disorder (Fan et al., 2016). HIV infection has the potential to alter cytokines and growth factors in microglia and astrocytes which may have direct consequences on hippocampal neurogenesis (Fan et al., 2016). Moreover, HIV has been shown to inhibit the proliferation of progenitor cells, thus directly affecting neurogenesis (Fan et al., 2016). Given the association of decreased neurogenesis with both depression and HIV, it is reasonable to suppose that much like dendritic spine alterations, neurogenesis decreases in both HIV and depression may

represent a common mechanism to both processes and thus a promising target for therapeutic intervention. However, few, if any, studies have directly examined the relationship between mechanisms of interaction between HIV, antidepressant medication, and neurogenesis.

Up until this point, the previous chapters have sought to provide an understanding of HIV and HIV-associated depression. As discussed, depression is a common comorbidity directly impacting treatment prospects for those individuals suffering from HIV, alongside HIV-associated neurocognitive disorders. Central to the present discussion of HIV and depression is the notion of dendritic spine dysfunction, which likely underpins many of the neurochemical and behavioral dysfunctions associated with both HIV and depression. Collectively, population shifts in dendritic spines to more juvenile states may potentially underlie and exacerbate many of the associated dysfunctions that have been discussed up until this point.

From this initial discussion of the history of dendritic spines and related spine dysfunction, attention was turned to discussing telomere length and doublecortin in the context of HIV and depression. Each of these phenomena likely plays a large role in the association of HIV and depression, although much less research has been done in these areas as opposed to the quite voluminous history of research concerning dendritic spines. Nonetheless, it is likely that comorbid HIV and depression involve the complex interplay of each of these phenomena which likely leads to the highly compromised neurological environment associated with both HIV and depression. With this in mind, the present research endeavor attempts to examine the relationship between each of the factors and how they may potentially be altered by antidepressant treatment.

As discussed, the HIV-1 Tg rat represents a non-infectious model of controlled HIV-1 protein exposure and is likewise an excellent rodent model for examining the present research questions. Escitalopram is a selective serotonin reuptake inhibitor (SSRI) that is commonly prescribed for depression and is recognized by the Veteran's Affairs Administration as a safe-accompaniment to cART treatment. Moreover, chronic escitalopram treatment has been previously reported to reverse chronic mild stress-induced cytogenesis decreases in the hippocampal dentate gyrus (Javatissa et al., 2006). Thus, escitalopram is a clinically relevant choice of medication to attempt to develop a potential treatment for HIV-comorbid depression.

The primary aim of the present research is to evaluate the effects of escitalopram treatment upon medium spiny neuron (MSN) proliferation in the nucleus accumbens of HIV-1 Tg rats. Research has consistently demonstrated anatomical and neurochemical impairment in this region using the HIV-1 Tg rat (Roscoe et al., 2014; McLaurin et al., 2018; Denton et al., 2019). Thus, the present research represents a first step toward establishing a therapeutic profile for escitalopram in the context of HIV and depression. The effects of chronic escitalopram treatment upon dendritic spine proliferation have been somewhat unclear up until this point, so the present research will hopefully address many of these gaps in the literature. A tertiary, but no less important focus of the present study is to evaluate the effects of chronic escitalopram treatment upon telomere length and DCX positive neuron count in the HIV-1 Tg rat. Unlike the previously discussed issue of dendritic spines, the precise relationship of both telomere length and DCX in the context of HIV, particularly in the HIV-1 Tg rat is much more unknown. Thus, in addition to providing a therapeutic profile for escitalopram treatment, the present study

will hopefully address many of the more basic scientific questions concerning the relationship between HIV, telomeres, and DCX.

CHAPTER 7

MATERIALS AND METHODS

7.1 ETHICS STATEMENT

Experiments were conducted in accordance with the recommendations listed in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The research protocols were approved by the Institutional Animal Care and Use Committee at the University of South Carolina (assurance number: D16-00028).

7.2 SUBJECTS

Animals (n=73; HIV-1 Tg=31, F344/N=42) were obtained from Envigo, (Indianapolis, IN) and pair housed under targeted conditions of 21°±2 °C, 50 %±10 % relative humidity with a 12-hour light: dark cycle. Animals were pair housed by both sex and genotype. Food (Pro-Lab Rat, Mouse, and Hamster chow # 3000) and water were available *ad libitum* throughout the duration of the experiment.

7.3 DRUG TREATMENT

Escitalopram (4 mg/kg) or placebo pellets (Innovative Research of America, Sarasota, FL) were subcutaneously implanted in the medial neck area of each animal. Animals were anesthetized using a 2-3% concentration of sevoflurane. Fur was removed from the target area and a small (approximately 3 mm) incision was made into which the pellet was placed. Incisions were then sutured, and each animal was administered a bodyweight dependent dose of butorphanol and placed in a recovery chamber with a heating pad. Animals were returned to their home cages after full locomotor recovery

occurred. Animals were monitored for one week in post-operative care. Animal body weight and well-being were monitored daily to ensure compliance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

7.4 ESTROUS CYCLE TRACKING

Vaginal lavage was performed on each day of the testing period to determine the cycle stage of female rodents. Each lavage was performed with approximately 1 mL of freshly prepared phosphate-buffered saline solution. The solution was administered to the vagina of the rat with a standard eyedropper and quickly retracted. The solution was then evaluated under a low-power light microscope to determine the cycle stage (Booze et al.,1999; Westwood, 2008).

7.5 PREPARATION OF TEZFEL TUBING

Tezful tubing (IDEX Health Sciences, Oak Harbor, WA) was cut and cleaned with a solution of polyvinylpyrrolidone (PVP) (EMD Millipore Corporation, Billerica, MA) and distilled H_20, and allowed to sit at room temperature before use.

7.6 PREPARATION OF DIOLISTIC CARTRIDGES

Cartridges were constructed as previously described (Roscoe et al., 2014). Briefly, tungsten beads (Bio-Rad, Hercules, CA) and crystallized Dil (Invitrogen, Carlsbad, CA) were dissolved in methylene chloride (Sigma-Aldrich, St. Louis, MO). Tungsten bead solution was applied to a standard glass slide before being treated with Dil solution and mixed until air-dried. The mixture was then removed from the slides and combined with distilled H_20 prior to probe sonication with a Branson Sonifier 150 (Branson Ultrasonics, Danbury, CT). The solution was then drawn into the previously prepared Tezfel tubing

and placed into a tubing prep station (Bio-Rad, Hercules, CA) for rotation until even distribution of the tungsten was achieved. The remaining liquid was drawn from the tubing with a syringe and nitrogen gas was blown through the tubing to ensure drying. The tubing was then cut into 13 mm segments and stored in a light-proof container.

7.7 BALLISTIC LABELING OF MEDIUM SPINY NEURONS

Animals (N=33) were sacrificed *via* transcardial perfusion of approximately 100 mL of freshly prepared paraformaldehyde approximately 6 weeks after having been implanted with escitalopram or placebo pellets. Brains were then removed and stored in paraformaldehyde. All terminal sacrifices of female rats were conducted during the diestrus phase of the rat estrous cycle. Brains were sliced on a standard rat brain matrix (Ted Pella, Inc., Redding, CA) at a thickness of 500 µm.

Five slices were taken from the nucleus accumbens of each animal and labeled with the Helios Gene Gun (Bio-Rad, Hercules, CA). Previously prepared cartridges were delivered at 70 psi through 3 um pore filter papers onto the tissue. Prepared slices were then washed with PBS and allowed to incubate at 4ºC overnight. The following morning, all tissue was mounted and cover-slipped with Fisherbrand 22X50-1.5 glass coverslips (Fisher Scientific, Pittsburgh PA)(Roscoe et al., 2014; McLaurin et al., 2018). Slices were imaged with a Nikon TE- 2000E confocal microscope (pinhole size 30 µm, pinhole projected radius 167 nm) using a green helium-neon laser with an emission of 533 nm (Nikon, Tokyo, Japan). Three neurons were imaged at both 20x and 60x magnification. 60x (n.a. = 1.4) images were traced for dendritic and spine complexity using NeuroLucida 360 (MBF Biosciences, Williston, TX). One neuron per animal was used to evaluate spine parameters using Neurolucida Explorer (MBF Biosciences, Williston,

TX). Dendritic spines were classified according to backbone length using an algorithm internal to Neurolucida 360 (Rodriguez et al., 2008). Length (µm), volume (μm^3), and head diameter (µm) were evaluated for each neuron. Spine lengths were defined as between .01 µm and 4 µm (Blanpied and Ehlers, 2004; Ruszczycki et al., 2012) while spine volume was measured between 0.02 μm^3 and 0.2 μm^3 (Merino-Serrais et al., 2013; McLaurin et al., 2018). Spine head diameter was defined as between 0.3 µm and 1.2 µm (Bae et al., 2012). A Sholl analysis was performed to examine dendritic complexity as measured by the number of intersections at successive 10 µm radii (Sholl, 1953).

7.8 EXTRACTION OF DNA FROM BLOOD SAMPLES

Approximately 2 mL of fresh blood was extracted from each animal during sacrifice and stored at -80º C until analyses are performed. Samples were then be thawed and allowed to warm to room temperature before being treated with a 2.5% heparin saline solution to prevent clotting. DNA extraction was performed using a Qiagen Dneasy Blood and Tissue kit (Qiagen, Hilden, Germany). Blood samples were combined with proteinase K and buffer AL and allowed to incubate in a 56º C water bath for ten minutes before being combined with ethanol and placed in a Dneasy spin column and collection tube. The mixture was then centrifuged at 8000 rpm for 1 minute. Buffer AW1 was then added to the spin column and the mixture was again centrifuged at 8000 rpm for 1 minute. Following the addition of buffer AW2, the mixture was centrifuged at 14,000 rpm for 3 minutes before being combined with buffer AE. The mixture was then incubated at room temperature for 1 minute before being centrifuged at 8,000 rpm for 1 minute. Extracted DNA samples were stored at 4º C. All materials necessary for this experiment were provided in the kit.

7.9 QUANTIFICATION OF TELOMERE LENGTH FROM DNA SAMPLES

Quantification of telomere length from extracted DNA was performed with an Absolute Rat Telomere Length Quantification qPCR Assay Kit (ScienCell, Carlsbad, CA cat#R8918). In brief, 20 µl mixtures of genomic DNA, nuclease-free H20, 2x qPCR master mix (Roche Applied Science, cat#06402712001), and either telomere primer (TEL) or single-copy reference primer (SCR) were prepared in standard PCR well plates. Samples were sealed and vortexed lightly. qPCR was performed with a DNA Engine Opticon 2 System for continuous fluorescence detection (MJ Research, Waltham, MA). Initial denaturation of the samples was performed at 95º C for 10 minutes. 32 cycles of denaturation, annealing, and extension were performed at 95º C, 52º C, and 72º C, respectively. Denaturation and annealing occurred for 20 seconds during each cycle while the extension was performed for 45 seconds. To quantify telomere length, ΔCq(TEL) and ΔCq(SCR) were calculated as the quantification cycle difference between the target sample and reference samples of DNA. ΔΔCq was then be calculated as the difference between ΔCq(TEL) and ΔCq(SCR). The relative telomere length of the target to the reference sample was calculated as $2^{-\Delta\Delta Cq}$. The total telomere length of the target sample was thus calculated as the reference sample telomere length multiplied by $2^{-\Delta\Delta Cq}$. All materials and calculation instructions were provided in the kit.

7.10 DOUBLECORTIN IMMUNOHISTOCHEMISTRY

Following transcardial perfusion with paraformaldehyde, rodent brains were extracted and sliced to 500 um using a standard rat brain matrix. Hippocampal slices (eight in total) were treated with a 1:1 solution of phosphate-buffered saline (PBS) and methanol with added H_20_2. Slices were allowed to incubate for 15 minutes before being

washed with PBS. A blocking solution consisting of Triton-X-100 and horse serum was then applied to the tissue, and the samples are allowed to incubate for 30 minutes before subsequent washing with PBS. Primary antibody (Doublecortin sc-271390 lot# C0218 Santa Cruz Biotechnology) was then applied to the samples which were incubated overnight at 4º C. The following day, slices were washed with PBS before a two-hour incubation with a secondary antibody (Biotinylated anti-mouse Santa Cruz Biotechnology). Following incubation and washing, tissue sections were mounted on standard microscope slides and allowed to partially dry before being treated with Vectastain ABC for 30 minutes. Sections were again washed prior to application of DAB. Once acceptable levels of staining are achieved, the sections were washed with distilled H20 to remove all DAB. Sections were then bathed in 70% ethanol for 2 minutes, 95% for 3 minutes, and 100% for 1 minute prior to a 3.5-minute xylene bath. Mounting solution (5:1 Permount: xylene) was then be applied and the sections were coverslipped.

7.11 STEREOLOGICAL METHODS

StereoInvestigator software (version 11) was used in conjunction with a Nikon Eclipse E800 microscope equipped with a motorized LEP MAC 5000 XYZ stage controller. The optical fractionator probe within the StereoInvestigator software allows for an unbiased estimation of the number of neurons and glial cells within the dentate gyrus. The region of interest was outlined at 4X magnification while all counts are performed with a 100X oil immersion objective (100x/1.45oil N.A.). Cell nucleoli were used as an object point for cell counting.

7.12 DATA ANALYSIS

All statistical analyses were performed using SAS (version 9.4), where a *p*-value of less than or equal to 0.05 was considered to be statistically significant. Spine frequency distributions were compared and graphed into histograms using GraphPad Prism (Version 5). Frequency distributional shifts for spine length were analyzed using a chi square analysis. Frequency distributional shifts for spine head diameter and volume were compared using a one-tailed likelihood ratio test for exponential distributions (Han et al., 2012). Sholl analysis was performed using Neurolucida Explorer to examine dendritic branching and complexity, then compared across genotype, sex, and treatment using a mixed model ANOVA (genotype X sex X treatment X intersections/radii). A discriminant function analysis was further used to analyze spine parameters obtained from Sholl analysis (SPSS version 24 and SAS version 9.4). A 2X2X2 ANOVA was used to evaluate differences in total spine counts between each treatment group. Separate 2X2X2 (genotype X sex X treatment) factorial ANOVAs were used to evaluate the main effects and interactions of genotype, sex, and drug treatment upon the dependent variables of telomere length and DCX positive neuron count, respectively. All graphs were produced with GraphPad Prism (version 5).

CHAPTER 8

RESULTS

8.1 DENDRITIC SPINE LABELING

Overall, MSNs of HIV-1 Tg animals treated with escitalopram exhibited greater dendritic length, volume, and intersections at distal radii, demonstrating that escitalopram was effective in promoting dendritic complexity and proliferation in the nucleus accumbens of HIV-1 Tg animals. Frequency distributions of spine length of medium spiny neurons in the nucleus accumbens revealed a genotype/treatment interaction effect with escitalopram altering length distributions for both HIV-1 Tg and F344/N animals as indicated by a chi square analysis [χ^2 (51)=1285.11, p≤0.001]. Though escitalopram did not appear to alter frequency distributions for head diameter or volume upon visual inspection, a one-tailed likelihood ratio test for exponential distributions (Han et al., 2012) revealed significant treatment effects for both measures [For head diameter: χ^2 (24)=396.62, p≤0.01; For volume: χ^2 (219)=651.10, p≤0.01]. Moreover, escitalopram appeared to alter spine morphology in HIV-1 Tg rats, as individuals treated with escitalopram exhibited higher frequencies of stubby and mushroom spine types across successive radii when compared with placebo-treated HIV-1 Tg animals. A Sholl analysis revealed a statistically significant interaction effect for treatment and genotype upon dendritic proliferation. HIV-1 Tg animals demonstrated markedly less dendritic complexity when compared with control counterparts. However, treatment with escitalopram served to dramatically improve dendritic complexity in HIV-1 Tg animals,

even normalizing animals to control levels with respect to dendritic intersections at concentric radii as indicated by a mixed model ANOVA [$F(1,8)=5.34$, $p\leq0.05$]. A similar effect was observed with respect to length and volume, although the effects were not statistically significant [$F(1,13)=2.18$, $p\geq 0.05$; $F(1,13)=1.51$, $p\geq0.05$, respectively]. Additionally, a discriminant function analysis with jackknife resampling procedure was performed to determine whether animals could be accurately classified into treatment groups (placebo vs. escitalopram) based upon dendrite intersections obtained from the Sholl analysis. Using the parameter of dendrite intersection/radii for each of the concentric radii, animals were correctly classified into treatment groups with 100% accuracy [Wilks' $\lambda=0.216$, $\chi^2_{(12)}=26.02$, $p \leq0.05$]. Finally, a factorial ANOVA examining spine by neuron counts revealed no statistically significant difference in total spine counts for each of the treatment groups, indicating that escitalopram-mediated spine shifts occurred independently of a global increase in spine totals [$F(1,22)=0.067$, $p\geq 0.05$].

8.2 TELOMERE LENGTH

A factorial ANOVA revealed no statistically significant differences in telomere length. HIV-1 Tg animals were not found to have shorter telomere lengths than F344/N controls, and no sex differences were found. Treatment with escitalopram did not serve to increase average telomere length regardless of genotype or sex. [$F(1,52)=0.54$, p=ns].

8.3 HIPPOCAMPAL DOUBLECORTIN

A statistically significant interaction between genotype and treatment condition was found using an ANOVA with escitalopram dramatically improving DCX positive neuron counts in F344 control animals. However, this effect was not demonstrated with HIV-1 Tg animals, which did not exhibit significant improvement despite treatment with

escitalopram, which suggests an important temporal aspect of escitalopram treatment which will be discussed in the following section [$F(1,13)=6.54$, $p < 0.05$].

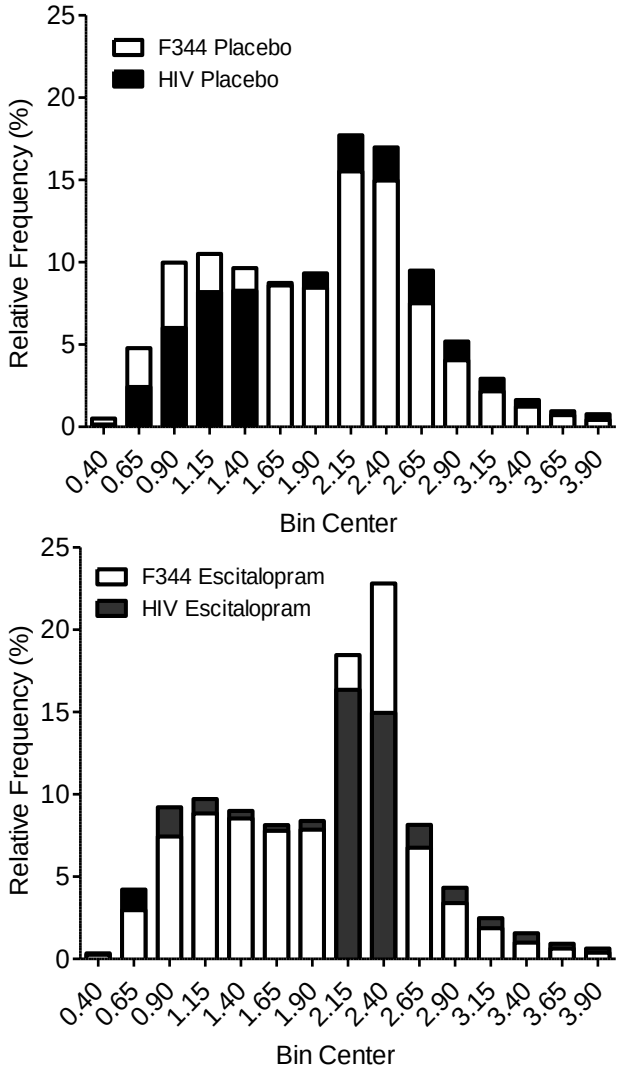

Figure 8.1: Frequency distributions of spine length in medium spiny neurons of the nucleus accumbens. Escitalopram treatment to both HIV-1 Tg and F344/N rats resulted in altered length distributions for each genotype condition. [χ^2 (51)=1285.11, p≤0.001].

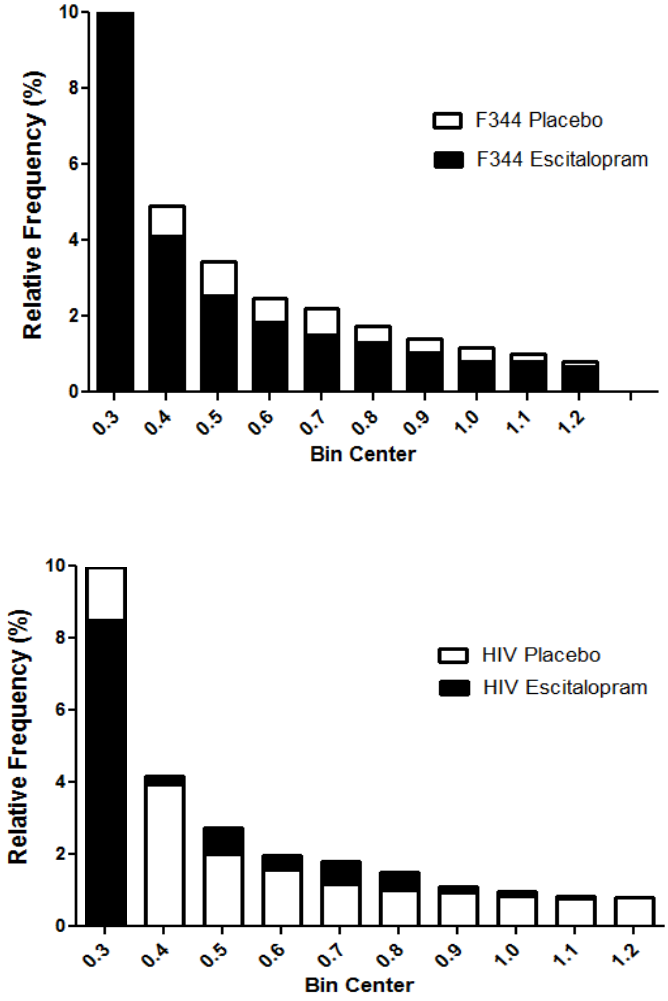

Figure 8.2: Frequency distributions of spine head diameter in medium spiny neurons of the nucleus accumbens. Escitalopram treatment altered spine head diameter distributions in both HIV-1 Tg rats or F/344 rats as indicated by a one-tailed likelihood ratio test [χ^2 (24)=396.62, p≤0.01].

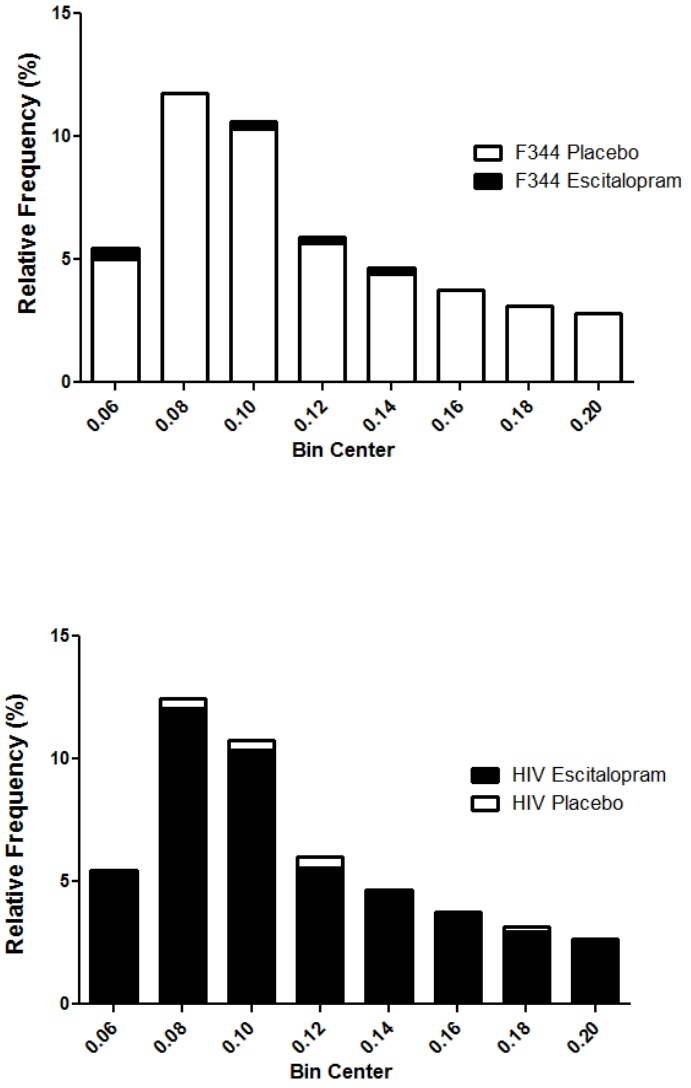

Figure 8.3: Frequency distributions of spine volume in medium spiny neurons of the nucleus accumbens. Escitalopram treatment altered spine volume distributions in both HIV-1 Tg rats and F/344 rats as measured by a one-tail likelihood ratio [χ^2 (219)=651.10, p≤0.01].

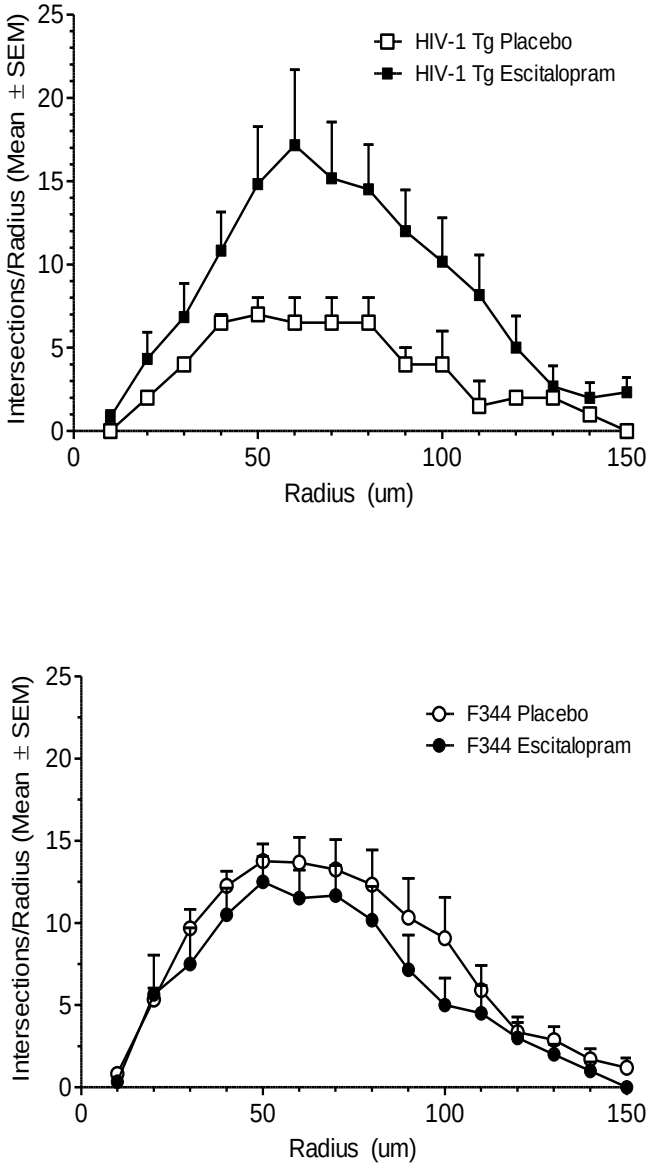

Figure 8.4: Dendritic intersections at consecutive radii. Overall, escitalopram treated HIV-1 Tg animals demonstrated significantly increased dendritic intersections at more distal radii, indicating an increase in dendritic proliferation ($p<0.05$).

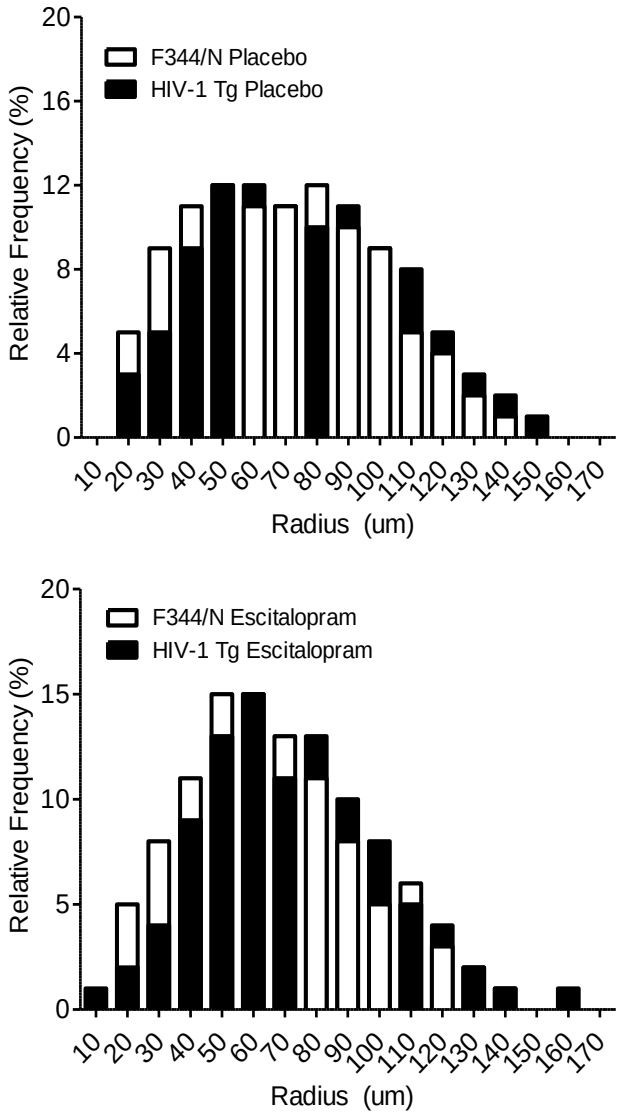

Figure 8.5: Relative frequency of mushroom spines treated with placebo versus escitalopram (top versus bottom) separated by genotype across consecutive radii. HIV-1 Tg animals treated with escitalopram demonstrated greater frequencies of mushroom spines at more distal radii.

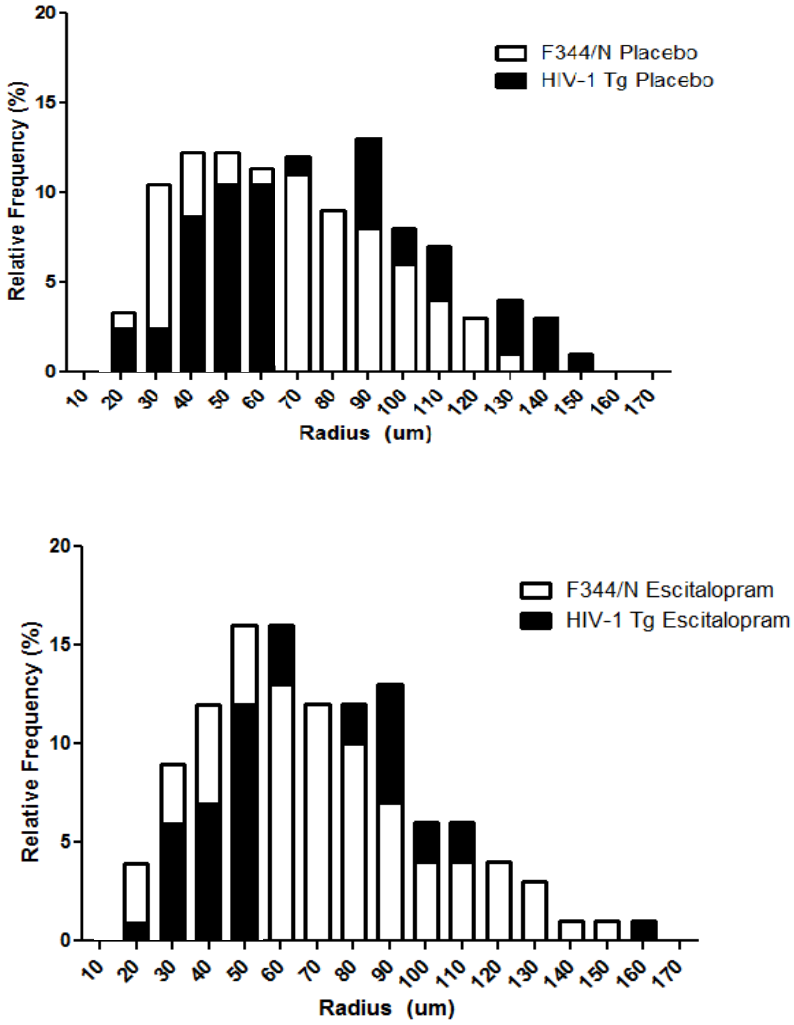

Figure 8.6: Relative frequency of stubby spines treated with placebo versus escitalopram (top versus bottom) separated by genotype across consecutive radii. HIV-1 Tg animals treated with escitalopram demonstrated greater frequencies of stubby spines at more distal radii.

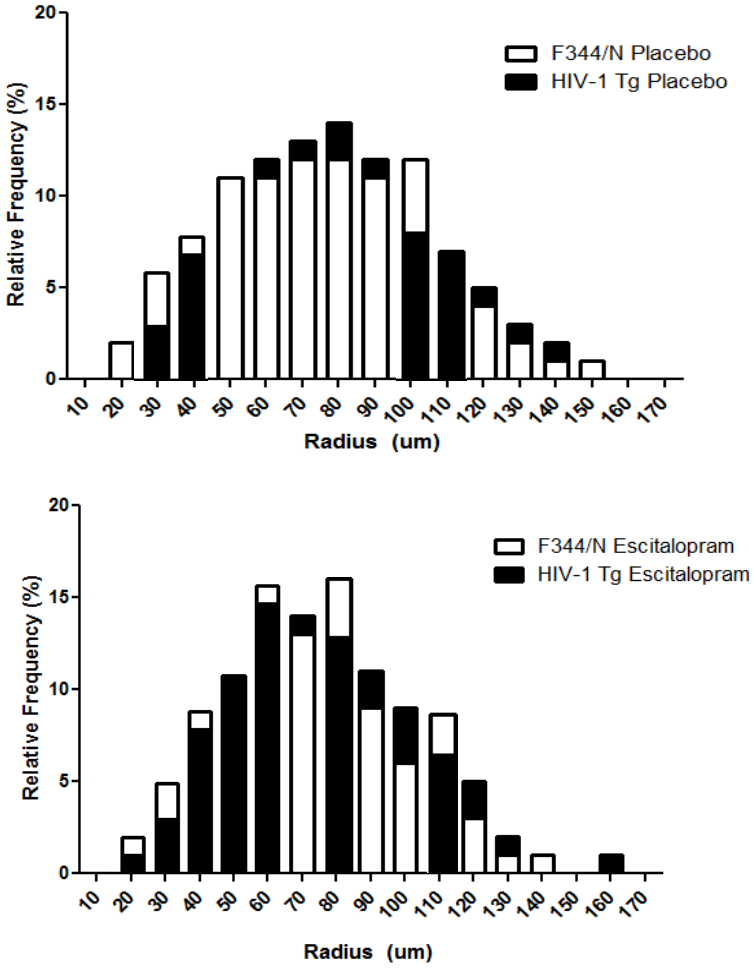

Figure 8.7: Relative frequency of thin spines treated with placebo versus escitalopram (top versus bottom) separated by genotype across consecutive radii. Globally, escitalopram treatment did not appear to alter frequency distributions of thin spines for either F344/N or HIV-1 Tg animals.

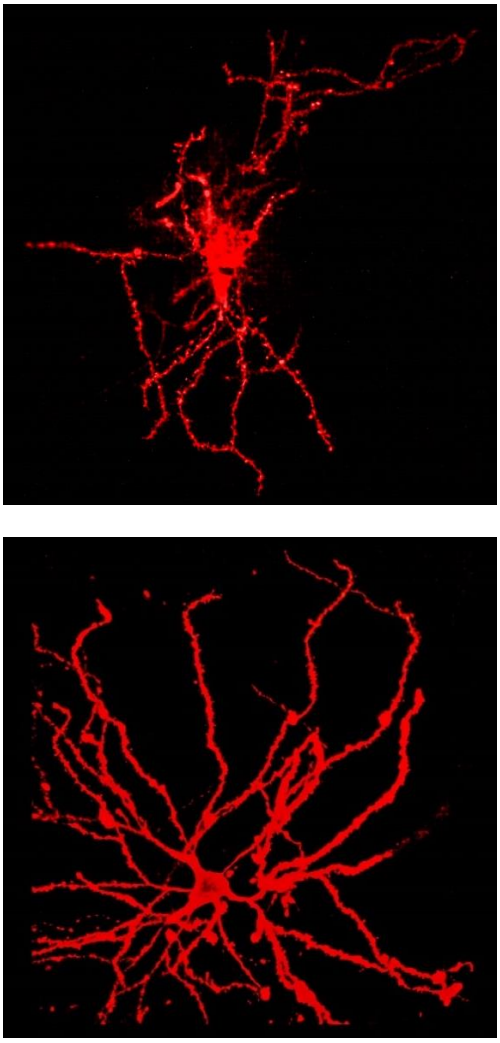

Figure 8.8: Representative HIV-1 Tg medium spiny neuron treated with placebo (top) vs HIV-1 Tg neuron treated with escitalopram (bottom).

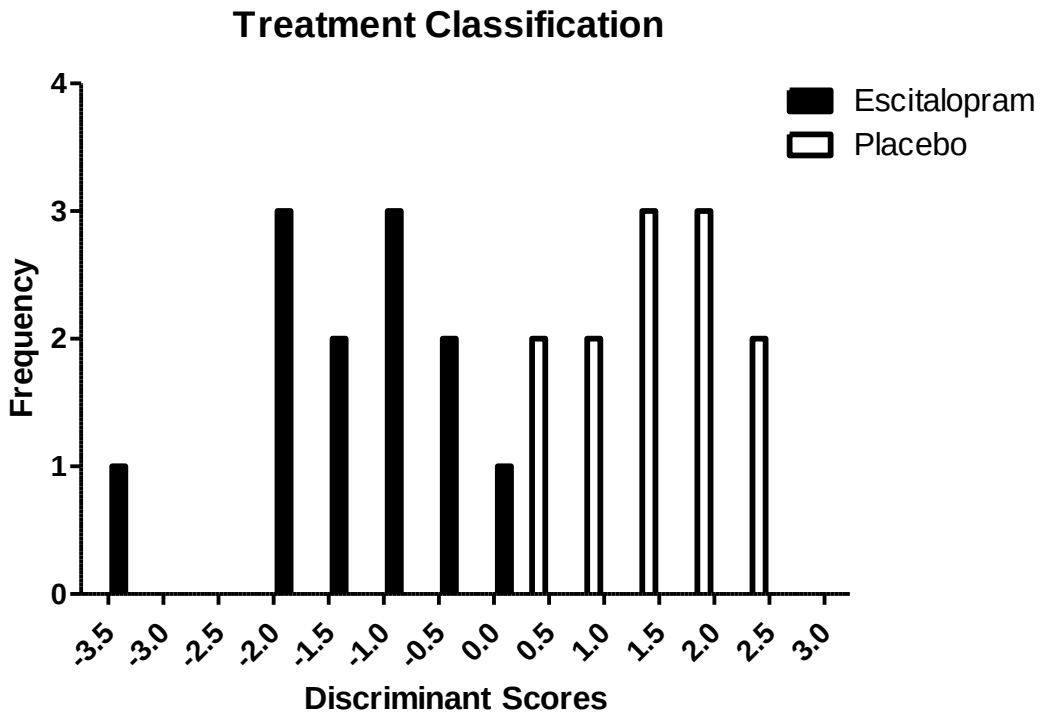

Figure 8.9: Histogram of discriminant function scores for each individual included within the present analysis. Using the parameter of dendrite intersection/radii for each of the concentric radii, animals were correctly classified into treatment groups with 100% accuracy ($p \leq 0.05$).

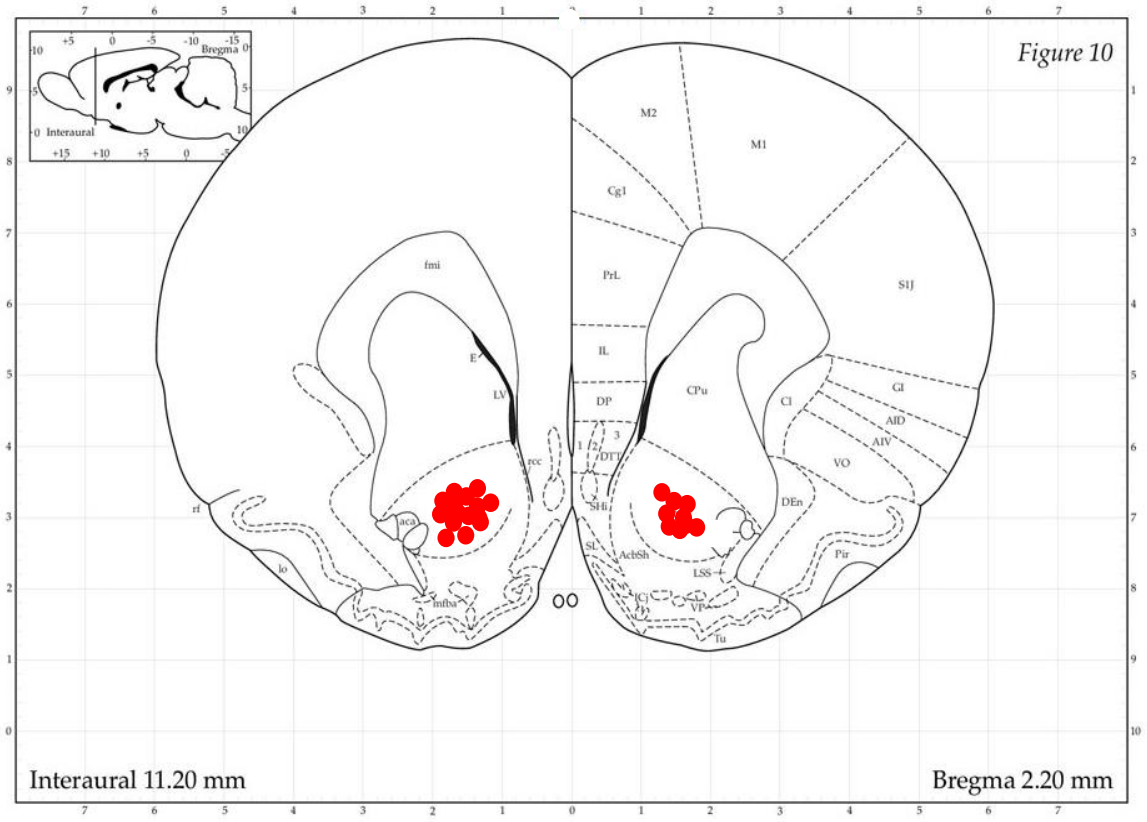

Figure 8.10: Medium spiny neuron locations of each animal examined in the present experiments.

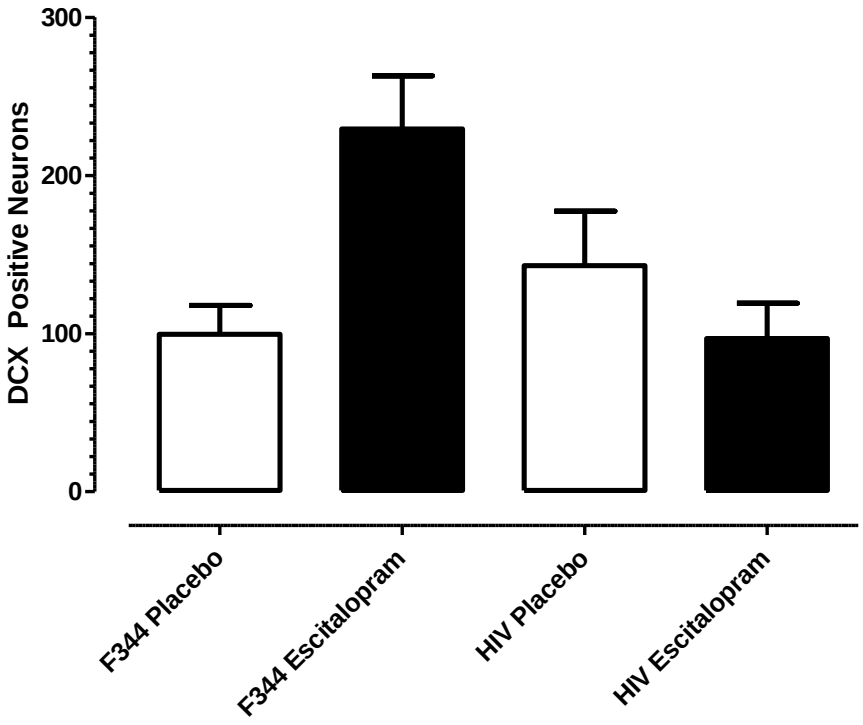

Figure 8.11: Doublecortin positive neuron counts in the hippocampus of HIV-1 Tg and F344/N rats. A statistically significant interaction between genotype and treatment was observed ($p<0.05$) with escitalopram treatment significantly improving doublecortin positive neuron count in the hippocampus of F344/N, but not in HIV-1 Tg rats.

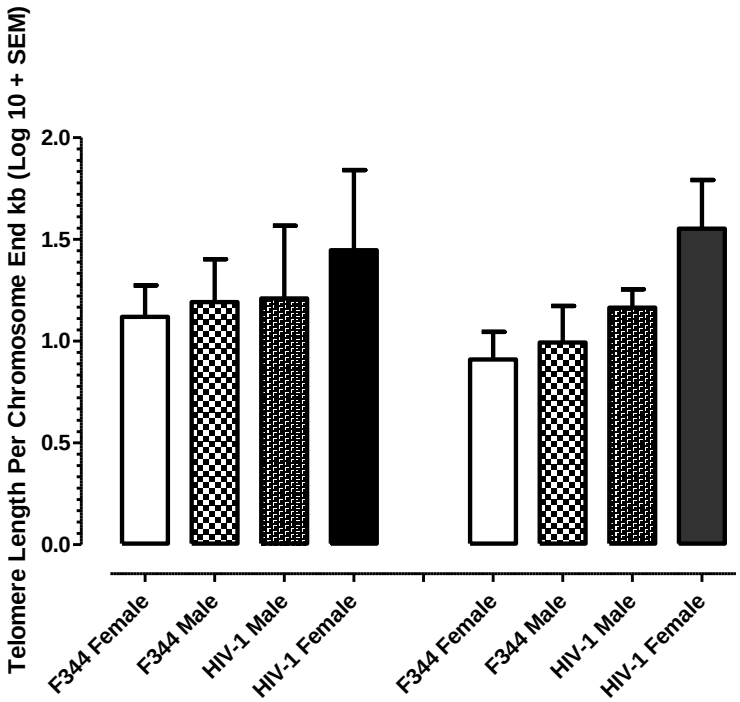

Figure 8.12: Average telomere length per chromosome end in each of the 8 conditions of the present experiment. Escitalopram (right group) did not increase average telomere length relative to placebo treatment (left group). Overall, no statistically significant differences in telomere length were found for any of the treatment conditions (p=ns). Though HIV-1 Tg females overall demonstrated the longest average telomere length, the effect was not statistically significant (p=ns).

CHAPTER 9

DISCUSSION

Chronic escitalopram treatment significantly increased dendritic complexity and altered spine morphology in the HIV-1 Tg rat. Previous reports found extensive HIV-induced damage to MSNs in the nucleus accumbens of HIV-1 Tg rats (Roscoe et al., 2014; McLaurin et al., 2018) thus, synaptodendritic restoration may be a key target for therapeutic intervention. We found that chronic escitalopram administration was successful in restoring dendritic complexity to MSNs in the nucleus accumbens of HIV-1 Tg rats, even to control levels. These findings suggest therapeutic efficacy for escitalopram in repairing HIV-mediated damage in the nucleus accumbens. However, escitalopram treatment did not increase hippocampal neurogenesis in HIV-1 Tg rats. Doublecortin positive neuron count in F344 animals was improved by escitalopram treatment; in contrast, HIV-1 Tg animals treated with escitalopram failed to display an increase in doublecortin positive neurons. Nevertheless, escitalopram treatment represents an important first step toward effective therapeutic intervention in repairing HIV-mediated synaptodendritic damage following exposure to HIV-1 proteins.

Synaptic loss, without neuronal death, is associated with HIV-1 and likely underlies neurocognitive impairments (Everall et al., 1999; Bellizzi et al., 2006; Ellis et al., 2009). As HIV-1 does not directly infect neurons, synaptic loss is a result of exposure to viral products such as HIV Tat and gp120 (Fitting et al., 2008). Specifically, the cysteine-rich region of the Tat protein has been shown to play a critical role in the

development of synaptic loss (Bertrand et al., 2013). Synaptic damage may be a result of Tat-produced proteasome-mediated degradation of micro-tubule-associated protein 2 (MAP2), which consequentially results in a collapse of cytoskeletal filaments and spine/synaptic loss (Kim et al., 2008). Whereas cellular death requires calcium-mediated neuronal nitric oxide synthesis, synaptic damage associated with Tat is not mediated by nNOS, but rather ubiquitin-proteasomal pathways (Aprea et al., 2006; Kim et al., 2008). HIV-induced synaptic loss may result from a compensatory process to avoid cellular death (Green et al., 2019), thereby altering circuit connectivity (Illenberger et al., 2020). HIV viral proteins and inflammatory cytokines in the brain result in excessive activation of glutamatergic pathways, particularly in the frontostriatal pathways, which are critical for apathy. Previous reports highlight the potential reversibility of HIV-1 induced dendritic damage, including the present report (Bellizzi et al., 2006; Kim et al., 2008; Kim et al., 2011; Bertrand et al., 2014). More research is needed to more fully elucidate effective treatments for HIV-1 induced synaptodendritic damage, although phytoestrogen treatment (Bertrand et al., 2014; McLaurin et al., 2020), cannabinoid receptor activation (Kim et al., 2011), and the presently discussed antidepressant treatment are promising treatment avenues. Moreover, functional endpoints of neurocognition are essential (McLaurin et al., 2019) in any assessments of neurorestorative treatments for HAND.

What is unclear from the present findings, however, is why escitalopram-mediated improvement in the dendritic spine profile did not produce a likewise increase in hippocampal neurogenesis. The present finding that escitalopram treatment increased mushroom spine proliferation, suggests the potential to rectify many of the deleterious effects of HIV-1. Mushroom spines are associated with the upper limits of synaptic

strength and represent mature synaptic connections (Yuste, 2010; Berry & Nedivi, 2017). Moreover, these spine subtypes have the largest spine head volume of all spine subtypes, which correlates to a larger pre-synaptic zone and post-synaptic density (Yuste, 2010; Berry & Nedivi, 2017). Increased size of the post-synaptic density is furthermore correlated with both the size of the active pre-synaptic zone and the number of docked pre-synaptic vesicles (Berry & Nedivi, 2017). Escitalopram treatment also increased in stubby spine subtypes. The stubby spine types have little to offer in the context of neurotransmission, as their shape and lack of a voluminous head do not engender effective neuronal communication when compared to spine subtypes such as thin or mushroom (Yuste, 2010). Though stubby spine populations are maintained in the adult brain, they are typically considered to be markers of incomplete synaptic development (Yuste, 2010; Berry & Nedivi, 2017). Moreover, increases in stubby spine proliferation are often associated with neuropathology, including clinical depression (Buyukdura et al., 2013). The findings that escitalopram increases both mushroom and stubby subtypes suggest that escitalopram treatment may be a first step toward repair of HIV-mediated damage in the nucleus accumbens, but likewise leaves several questions unanswered.

The most likely explanation for why an increase in doublecortin positive neurons was not observed is the timing of the present investigations, coupled with the likelihood that neurogenesis occurs slower than the formation of dendritic spines. Such a hypothesis would explain why escitalopram produced an increase in spine subtypes conventionally associated with immaturity while likewise increasing populations of those types that represent the upper limits of synaptic strength. Future studies of chronic escitalopram may show increased neurogenesis if measured at longer times from treatment onset.

Thus, while escitalopram has the potential to dramatically increase dendritic branching and spine proliferation within six weeks of treatment, SSRI therapies may require a longer treatment period to reach full effect. Nevertheless, spine loss in the context of HIV-1 can be recovered by therapeutic intervention. Full recovery of circuit potential and neurogenesis are likely a process that takes longer than changes in dendritic spines, thus a longer investigation may observe the full restoration of circuit connectivity and increased neurogenesis following treatment with escitalopram. Such a result would be in line with current lines of research within the field, as neurogenesis has been associated with the effects of SSRI medication (Micheli et al., 2018).

The present experiments did not find a statistically significant difference in average telomere length between HIV-1 Tg and F344/N rats or between rats treated with placebo or escitalopram. Independent of treatment or genotype, average telomere length was consistent between each treatment group. Interestingly, HIV-1 Tg female rats displayed the longest average telomere length regardless of treatment condition. Whether or not this suggests a potential sex by genotype interaction with respect to telomere length is unclear and perhaps not answerable by the present research, as this finding was not statistically significant, but may serve as an interesting research question for future deliberation.

Telomeres are present at the end of chromosomes and protect from chromosomal degradation and recombination (Vakonaki et al., 2018). Telomere degradation is a constant feature of many inflammatory conditions including depression and HIV (Vakonaki et al., 2018; Auld et al., 2016). While telomere shortening is a natural process associated with cellular aging and thus repeated cellular division, factors such as

inflammation and oxidative stress can expedite the telomere shortening process. Oxidative stress is particularly harmful to telomeres, which results in the formation of 8-oxo-7,8dihydro-2'deoxyguanosine formation at the GGG triplet of the 5'TTAGGG-3' telomere sequence, which is subsequently cleaved by formamidopyrimidine-DNA glycolyase, resulting in the loss of large telomere sections during cellular division (Zhang et al., 2016). Thus, inflammation and subsequent oxidative stress has the direct consequence of driving down telomere length, thus altering cell senescence. Presently, telomere shorting was not reported in HIV-1 Tg rats, which may suggest an overall lack of inflammation in the model of HIV, at least detectable by the present analysis or in HIV-1 Tg rats of advancing age. Longitudinal work examining telomere attrition in the HIV-1 Tg rat may prove to be invaluable to gain an understanding of the time course of inflammation and telomere length in rodent models of HIV.

Several lines of research have argued that transgene-induced protein production leads to increased inflammation in HIV-1 Tg rat (Royal et al., 2007; Homji et al., 2012; Royal et al., 2012; Vigorito et al., 2015). However, few if any studies have directly examined the effects of the HIV-1 transgene on telomere length. Presently, it is thus difficult to evaluate these findings against the overall lack of literature concerning the topic. However, one key limitation of the presently used methods is that rodent blood used for these analyses was removed from intact HIV-1 Tg rats during sacrifice. Perhaps a much clearer picture of telomere dysfunction in the HIV-1 Tg rat could be elucidated by examining more specific cell lines exposed to viral proteins rather than using the broad approach that was presently employed. Indeed, by examining specific cell lines in cellular culture exposed to HIV viral proteins and subsequently measuring telomere

length, perhaps a more selective understanding of telomere dysfunction in HIV can be elucidated.

Antidepressant adherence and telomere length likewise suffer from a considerable lack of examination in the literature. Few studies have examined the relationship between antidepressant adherence and telomere length, though evidence has been presented that telomere length may be somewhat predictive of antidepressant treatment response (Rasgon et al., 2016). Few if any subsequent reports have followed up on these findings, with the specific relationship between antidepressant medication and telomere length remaining elusive. Presently, escitalopram was not found to attenuate telomere length. Future research endeavors should likely examine longer treatment periods, however, as it is unclear the exact timing of medication adherence that would be required to produce meaningful change. Regardless, the present state of the scientific literature is likely several years away from a firm understanding of the relationship between antidepressant treatment and telomere morphology.

CHAPTER 10

CONCLUSIONS AND FUTURE DIRECTIONS

Similar to the presently discussed antidepressant treatment, phytoestrogen treatments have been shown to be a promising potential therapeutic for HIV-1 induced neuroanatomical damage. Phytoestrogen compounds are plant-derived compounds that are found to partially mimic estrogen in mammals. Pretreatment of cells with the phytoestrogen liquiritigenin (LQ) has been demonstrated to be protective against Tat-mediated F-actin loss (Bertrand et al., 2014). Filamentous actin (F-actin) is a major cytoskeletal protein that comprises synaptic structures such as dendritic spines. Polymerization of globular actin (G-actin) into F-actin is a crucial step that occurs prior to spineogenesis (Johnson et al., 2006). F-actin is found in both the head and shaft of dendritic spines in addition to non-spiny synapses (Johnson et al., 2006). Estrogens have been previously demonstrated to modulate concentrations of F-actin in dendritic spines, thereby acting as a potential therapeutic that may promote plasticity (Sanchez et al., 2009).

Cannabinoid receptor activation has been demonstrated to inhibit gp-120 induced synaptodendritic damage in cell cultures of hippocampal neurons (Kim et al., 2011). 24-hour treatments of gp120 to cultured cells of the hippocampus result in a concentration-dependent decrease in the number of neuronal puncta by approximately 37 percent (Kim et al., 2011). Specifically, gp120 activates the CXCR4 receptor on microglia to produce the release of interleukin-1B (IL-1B). Sequential activation of IL-1B receptors and N-

methyl-D-aspartate is further required to produce synapse loss, which as previously described, precedes apoptosis (Kim et al., 2008; Kim et al., 2011). Treatment with the cannabinoid receptor agonist Win55212-2 inhibited gp120 mediated synapse loss. Further experimentation in the same study demonstrated that the protective effects of Win55212-2 were mediated through the CB2 receptor (Kim et al., 2011). Specifically, Win55212-2 inhibited gp120 induced IL-1B production from microglia, thus reducing the overall inflammatory environment. Thus, cannabinoid-based treatments may have the potential to be a therapeutic option for HIV-seropositive individuals. The increasing availability of cannabis products coupled with increasing legalization at the state level may promote the use of cannabis products for HIV positive individuals, though more research in both intact animal models and clinical trials are needed to fully flesh out potential efficacy. Given the relatively low abuse potential of cannabis, however, this avenue should not be ignored.

As NMDA receptor activation is central for gp120-mediated synaptodendritic damage in HIV-1, a logical candidate for potential treatment are NMDA receptor antagonists. Although this is a very logical candidate for adjuvant HIV-1 therapies, studies have failed to indicate the efficacy of these compounds in reducing the manifestations of HAND. NMDA antagonists (such as memantine) have been investigated as a potential adjuvant therapy to cART but do not appear to have any dramatic effect upon the manifestation or intensity of HAND (McGuire et al., 2014). Similarly, GABAergic-based interventions such as valproic acid have likewise failed to produce alterations in HAND (McGuire et al., 2014). Although activation of NMDA receptors is necessary for gp120-mediated synaptodendritic damage, it is clear that

perhaps the optimum therapeutic for HIV-1 would occur earlier along the pathway, perhaps at the level of microglia and IL-1B expression.

Speaking to the notion of compounds that support plasticity, serotonin-like psychedelics such as psilocybin, lysergic acid diethylamide (LSD), and ketamine have been demonstrated to promote both spineogenesis and neurogenesis on an incredible scale. Mature rat cortical neuron cultures treated with LSD for 24 hours showed increased dendritic spines per length unit, with LSD in particular nearly doubling the number of spines per 10um length segment (Ly et al., 2018). Similar effects were found with respect to an intraperitoneal dimethyl tryptamine (DMT) injection into intact rats. While these serotonin-like psychedelics do indeed produce significant increases in spine density mediated by VGLUT1 puncta, shifts in spine morphology following psychedelic treatment appear to consist more-so of immature spine types such as thin and filopodium, rather than mushroom spine types (Ly et al., 2018). Studies involving the HIV-1 Tg rat have consistently demonstrated shifts toward immature spine types accompanying HIV (Roscoe et al., 2014; Denton et al., 2020). Thus, it is unclear whether or not such treatment would be beneficial in combating HIV-mediated synaptodendritic damage. These compounds do deserve mention here, however, as current FDA mandates have increased the availability of such compounds for research purposes, though specific research with HIV models will be necessary to establish a consistent therapeutic profile.

Overall, it would appear that the gradual breakdown in reward memory underlies the development of apathy and anhedonia (motivational dysregulation) in both HIV and depression, and is likewise a common denominator in the frequent comorbidity of these conditions. This breakdown is not necessarily a complete elimination of a memory trace,

but rather the progressive decrease in memory salience of reward as motivational dysregulation progresses. As apathetic and anhedonic symptoms persist, previously positive memory traces that would promote and engender motivation to engage in once desirable behavior become gradually weakened as a function of neural rewiring in reward centers of the brain. This neural rewiring is reflected in the previously reviewed literature, as the proliferation of less mature spine types (associated with lower synaptic function) are consistently found in conditions that present with apathetic and anhedonic symptoms, including HIV-1 and depression. Thus, synaptic rewiring to more juvenile states of plasticity may account for the gradual progression of anhedonic and apathetic symptoms, which coincide with a decrease in the saliency of reward-related memory which is underpinned by the loss of mature synaptic connections and consequent loss of dopaminergic function (Roscoe et al., 2014; Denton et al., 2019; Denton et al., 2021).

As an important note, many of the reviewed articles discuss spine dynamic changes in terms of increases or decreases in the proliferation of a particular spine subtype. However, this language is insufficient to adequately categorize the full nature of changes in spine dynamics. Describing spine shifts in such a way does not fully describe the relationship between spine changes and synaptic rewiring. To this effect, a more appropriate description of spine changes is a shift in spine population in terms of morphology, not a simple increase or decrease in a particular morphological subtype. Under normal conditions, histograms of spine populations are somewhat right-skewed with long tails and moderate kurtosis (Yuste, 2010). Under pathological conditions, however, such population shifts are characterized by greater right-skewness and increased kurtosis as a function of the proliferation of less mature spine types.

Such population shifts can most accurately be measured by morphological features such as spine head volume or total spine volume. Thus, a more global population-based model of spine dysfunction in motivation dysregulation shows leftward (right-skewed) histograms of spine volume, both head and total volume, which is reflective of the proliferation of less mature spine subtypes which are typically less voluminous. Characterizing the spine changes associated with motivational dysregulation in this manner provides a more holistic, population-based account of spine dynamic changes which engender more efficient discussions of synaptic integrity. Moreover, simply describing spine changes in terms of simple increases or decreases in a particular subtype of dendritic spines is quite problematic given that spine morphologies exist on a continuum. This has led some authors such as Yuste (2010) to suggest that the traditional categorization scheme proposed by Kaiserman Abranoff (1970) is insufficient to adequately characterize spine morphology. The presently proposed population-based account of spine changes that accompany motivational dysregulation is above this criticism, however, as one need not consider more traditional classification schemes in such a population-based account.

As previously stated, the most important morphological features of dendritic spines to consider under this population-based account are spine head volume and spine total volume. Measures such as spine neck length appear to be differentially regulated from spine head volume (Yuste, 2010). Moreover, spine neck length does not appear to be associated with the size of the postsynaptic density, or the capacity for neural transmission (Yuste, 2010). Both spine head volume and total volume, on the other hand, are positively correlated with the size of the post-synaptic density. These measures are

positively correlated with the size of the pre-synaptic zone, which, in turn, influences the number of presynaptic receptors and docked vesicles. Thus, the consequence of population shifts toward less mature spine types associated with apathy and anhedonia has the direct consequence of reducing both the pre-synaptic zone size and the size of the post-synaptic density. This, in turn, leads to fewer docked vesicles and receptors which results in an overall downregulation of the synaptic structure. The net result of this process is an overall breakdown in synaptic strength. This breakdown in synaptic strength may perhaps undercut the previously discussed decreased saliency of reward-related memories.

Globally, the net result of the changes described is a breakdown in synaptic integrity and thus a breakdown in the reward saliency of reward-related memories. This process may indeed be mediated by the gradual progression of anhedonic and apathetic symptoms, which, as described previously, work in a cycle of feedback to degrade motivational behaviors. Population shifts in dendritic spine morphology under these conditions are characterized by an increase in thin spine populations. These spines are less mature and possess decreased head volume and total spine volume relative to more mature spine subtypes such as mushroom. The immediate consequence of this is a reduction in postsynaptic density size and the size of the presynaptic zone, which is accompanied by a decrease in docked receptor and vesicle count. Globally, these features account for the decreased capacity for transmission and thus decreased synaptic integrity that characterizes these synapses.

Returning to the discussion of therapeutic avenues regarding spine dysfunction, several lines of reasoning have suggested that therapeutic avenues for treating conditions

associated with compromised synaptodendritic integrity should be focused upon stabilizing populations of dendritic spines. While this idea is very attractive, it likewise carries a cautionary note. While stabilizing spine population numbers may be possible through any number of pharmacological interventions, there is not a concise picture of what "stable" means in this context. Indeed, some authors such as Yuste (2010) have argued that dendritic spine populations, under normal functioning, work in concert to ensure a high degree of synaptic connectivity and ensure an optimal level of functional connectivity. Computationally speaking, treatments designed to increase dendritic spine population numbers in a specific location may carry the unintended consequence of deleteriously affecting synaptic function, both inside the location of interest and globally within the brain as a whole. As illustrated by the present discussion "more" spines is not necessarily "better". Thus, treatments designed to this end likely will carry unintended consequences. There is an incredible and beautiful natural variation in spine morphology and population that characterizes the brain under both normal and pathological functioning. It is unclear at this juncture if selective alterations in these populations and morphological would be tantamount to improving functioning across neuropathological conditions. Moreover, there are significant gaps in the literature in terms of the overall relationship between dendritic spine pathology and synaptic function. Findings along these lines both in respect to normal functioning and pathology can often be seemingly disconnected and are somewhat contradictory in many cases. Speaking directly to this concern, the final discussion point of this discussion will suggest potential improvement to research paradigms involving synaptodendritic pathology, and provide suggestions for future research with escitalopram in HIV.

Even a cursory glance at the literature surrounding dendritic spines reveals a significant lack of computational models for global synaptodendritic function. Many research endeavors only examine alterations in one or a few specific locations of interest but fail to account for the ramifications these alterations have upon global neurological functioning. To this end, the tools provided by computational neuroscience may offer a solution to this issue. There is a paramount necessity within the literature for global models of functioning, both under normal and pathological circumstances. Tools such as network analyses and stochastic modeling would offer incredible insight towards developing a more global understanding of neural function. Unfortunately, few studies (none reviewed presently) have employed these computational tools to this end. Of course, such a research prospect would involve significant cross-disciplinary collaboration. Indeed, the combination of many powerful tools from divergent fields such as statistics, computer science, and mathematics could provide valuable insight toward this goal. Across the literature presently reviewed, there seems a significant fear of neuroscientists to step outside conventional ANOVA design frameworks and employ more complex yet powerful tools.

Speaking to this idea, present descriptions of spine morphologies and categorizations as “thin”, “stubby” and “mushroom” often fail to capture that incredible variation expressed in dendritic spine morphology (Yuste, 2010). While these categorizations do indeed represent useful shorthand in discussing spine dynamics and have played a critical role in understanding up to this point, the current status of the literature suggests that it is time for a critical reappraisal of dendritic spine variation and perhaps an update of definitions. More globally, future research endeavors should focus

upon embracing this natural variation and its implications for neurological functioning, rather than using conventional definitions of classification that may not capture the fundamental variation displayed by dendritic spine populations. Furthermore, this suggests that many of the algorithms used to classify and analyze dendritic spines should be updated to more accurately reflect this variation. This suggestion is in line with concerns that have been previously raised (Yuste, 2010). Extending this notion, future studies should likewise place greater emphasis on physiological features of dendritic spines such as receptor kinetics and post-synaptic densities, rather than employ more traditional classification schemes.

Next, studies of dendritic spine populations and morphologies should not be divorced from complementary investigations of neurochemical functioning. Indeed, modern neuroscience has a litany of tools at its disposal to accurately examine neurophysiological function. Such tools include the previously discussed fast-scan cyclic voltammetry in addition to tools such as single-cell recording and microdialysis. By combining examinations of dendritic spine populations with complementary examinations of physiological functioning, more comprehensive results can be obtained. Moreover, the conclusions obtained from these results are likely to produce a significantly increased understanding of spine pathology and neurological/neurochemical functioning. On a similar note, few studies, if any have examined pre-synaptic function associated with HIV- mediated morphological changes in HIV-1. Denton (2021) found that restoration of dendritic branching and morphological shifts in spine populations occurred independently of increases in dopaminergic or serotonergic transmission. While the most clearly plausible explanation for this concerns timing of the treatment, perhaps

investigation of pre-synaptic function in terms of vesicle functioning and calcium transmission would shed additional light on the relationship between spine dysfunction and neurotransmission in HIV-1.

As a final recommendation for future research involving dendritic spines, examinations should consider studying filopodia counts as a potential biomarker of spineogenesis. Dendritic filopodia are thin, hair-like appendages on the bodies of dendrites that lack a clear spine head/neck differentiation. While filopodia have no clear synaptic density with few vesicles, several authors have suggested that filopodia represent the earliest stages of spine development and account for 10% of the dendritic spine volume in the adult brain (Berry and Nedivi, 2017). While it is often argued that filopodia should be excluded from investigations of dendritic spines, establishing exclusion criteria is no trivial task, as it is often difficult to distinguish filopodia from thin spines without the aid of advanced techniques (Berry and Nedivi, 2017). Furthermore, owing to the composition of filopodia, they can be difficult to label with conventional staining techniques.

The case for inclusion of filopodia into conventional studies of dendritic spines hinges largely upon their hypothesized function within the nervous system. Several authors have hypothesized that filopodia are an important step in spineogenesis, particularly in pyramidal and Purkinje cells (Yuste, 2010; Berry and Nedivi, 2017). Dendritic filopodia have many features in common with other filopodia in the nervous system, which has led researchers to further suggest a potential role in dendritic spine development (Berry and Nedivi, 2017). Under this protospine model of spineogenesis, protospines are hypothesized to be intermediate dendritic structures emerging from

filopodia that gradually become stabilized throughout the developmental period. Upon establishing connection with a viable axon contact, these filopodia create a virtual dendrite that produces an exploratory area for developing spines in a similar fashion to how axonal connections are established during early development (Yuste, 2010; Berry and Nedivi, 2017). As one final piece of data in support of this model of spineogenesis, dendritic filopodia have considerable actin composition in the cytoplasm, which has been proposed to drive early dendritic connections (Yuste, 2010).

The obvious issue in the present case with this account of filopodia hinges upon the earlier statement that they are incredibly difficult to label using conventional techniques. Indeed, the actin composition of these structures makes it very difficult to label filopodia with conventional techniques such as the Golgi stain, and immunohistochemical preparations more broadly as filopodia do not respond to fixatives owing to their composition. However, this issue may be circumvented by employing *in-vivo* imaging techniques. By using such techniques, perhaps a more global understanding of the mechanisms behind dynamic spine changes may present itself, which would be critical for the future of broad investigations of dendritic spine populations.

Regarding the future of escitalopram usage in the treatment of HIV and comorbid depression, the present findings suggest several logical progressions that research should take. First and foremost, longer treatment times with escitalopram should be examined. While six weeks of continuous escitalopram treatment was effective in increasing dendritic complexity and mushroom spine populations, a likewise increase in neurogenesis in the hippocampus was not observed. As previously discussed, hippocampal neurogenesis is a hallmark feature of antidepressant efficacy. Though, in the

present study, escitalopram mediated neurogenesis was not found, although synaptic rewiring was firmly demonstrated. The most likely explanation for these seemingly divergent findings is timing. Synaptic reorganization in the form of dendritic spine remodeling is a relatively fast process compared to neurogenesis, which is markedly slower. Perhaps an examination of escitalopram treatment over a greater time period would indeed yield an increase in hippocampal neurogenesis. To this end, a continuation of this line of research should logically begin with an examination of longer treatment times. Given the well-documented variability and instability of antidepressant medications, treatment times of 2-6 months would not be unreasonable to answer the questions that the present research has posed. Six weeks of treatment is likely too short of a time period to see gravitational escitalopram mediated changes.

The second suggestion for future research, which should perhaps occur simultaneously with the first, is to investigate variable dosages of escitalopram. The presently discussed research elected to use a 4mg/kg dosage of escitalopram in an attempt to establish a baseline therapeutic efficacy of escitalopram. While 4mg/kg is a clinically relevant dosage of the drug and is a dosage that is routinely prescribed in the course of depression treatment, it is a rather low dose of the drug. Given the variability in response to antidepressant medication it is a logical extension of the present findings to attempt to test potentially greater dosages. Indeed, higher dosages of antidepressants, in particular, SSRI's are often employed on a case by case basis in clinical practice. In addition to the practical implications for examining the effects of variable dosages, such research could establish a dose-response curve for potential treatment which would no doubt be valuable information.

Indeed, the present findings are a first step in potentially elucidating the full therapeutic efficacy of escitalopram. By establishing both a dose-response relationship and time-course of treatment, escitalopram treatment in the context of comorbid HIV and depression could be tailored to meet situational and individual requirements for treatment and be administered in a more effective fashion. As described in the beginning chapters of the present discussion, clinical depression is one of the most frequent and most detrimental comorbidities associated with HIV and HAND. Comorbid depression has the potential to significantly alter treatment outcomes for individuals affected with HIV. While a casual search of available scientific resources has indicated that many researchers are indeed aware of this issue, most of the literature surrounding HIV comorbid depression consists of either qualitative descriptions, quantitative epidemiological measures, or literature reviews. Shockingly few studies have sought to address the problem at some fundamental level, whether it be preclinical investigations or clinical trials. The impetus is now placed upon the scientific community to elucidate and develop effective therapies to combat this issue. By establishing dose-response and treatment time course measurements of antidepressant (not just escitalopram) therapy in cases of comorbid HIV perhaps measurable progress towards decreasing problems associated with HIV may be made.

Continuing this discussion, an additional focus of research efforts going forward should evaluate if antidepressants, not just escitalopram, are truly safe accompaniments to cART therapy. While the Veteran's affairs administration recognizes escitalopram as a safe accompaniment to cART, many recommendations are not available for other commonly prescribed antidepressants. Every effort to ensure adequate adherence to

antidepressant and cART mediation in cases of comorbid HIV and depression should be made to ensure to a most beneficial outcome for the patient in question. At the present moment, it is unclear whether certain types of antidepressant medication may have any sort of harmful effect when mixed with cART treatment.

Future research endeavors likewise should consider the use of dopaminergic agonists in the treatment of comorbid HIV and depression. Given the previously described neurobiological underpinnings of apathy/anhedonia and HIV, a dopaminergic agonist is a logical choice of treatment, particularly when combined with an SSRI. Indeed, the combination of dopaminergic agonists and conventional antidepressant medication is not a novel suggestion and has been tried to some success in clinical practice (Belujon and Grace, 2017). Such procedures have demonstrated success in combining SSRI medication with more mild antipsychotic medications (Belujon and Grace 2017). Although, as a caveat to the present discussion, such procedures have never been tried in the context of comorbid HIV and depression, so it remains unclear if such strategies would remain successful in the presence of significant HIV mitigated dopaminergic disturbance.

To conclude the present discussion, HIV is a serious viral infection that affects a significant percentage of the global population. Although the development and proliferation of cART medication have dramatically increased the prospects for living and coping with HIV, significant cognitive and emotional disturbances remain present. Cases of HAND and cases of comorbid depression present themselves at an approximate rate of 50% despite adherence to cART. Most disturbingly, rates of suicide are significantly high in the HIV seropositive population suffering from depression.

Relatively few studies have attempted to suggest and examine potential treatments for HIV-1 comorbid depression. Potentially underlying both depression and HIV are significant changes in the morphology and population numbers of dendritic spines. Dendritic spines are key synaptic features for communication. Altering the morphology and population of these structures has the consequence of severely disrupting synaptic communication. Such alterations may potentially explain the deficits in dopaminergic and serotonergic transmission repeatedly observed in both HIV and depression. Synaptodendritic damage in HIV is a process that is distinguishable from cellular apoptosis through a similar but crucially unique biochemical pathway. In addition to being a potential neuroanatomical underpinning of HIV comorbid depression, synaptodendritic damage in HIV has been shown to underly many of the symptoms of HAND, though such synaptic damage may be compensatory in nature and protect the system from cell death via glutamatergic excitotoxicity.

The present set of experiments evaluated the therapeutic efficacy of escitalopram treatment to the HIV-1 Transgenic rat. Though escitalopram did not show to improve either markers of neurogenesis or telomere length, the treatment dramatically restored dendritic complexity in medium spiny neurons of the nucleus accumbens. Additionally, escitalopram treatment resulted in statistically significant shifts in spine morphology, with SSRI treated HIV-1 Tg rats demonstrating an increase in both mushroom and stubby spine subtypes. While the present experimental endeavors leave many potential research questions and considerations of the broad relationship between HIV, depression and spine damage, the present findings nonetheless suggest escitalopram to be a potentially beneficial therapeutic in the treatment of comorbid HIV and depression.

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