Involvement of the Cortical Cholinergic Receptor System in Symptoms of Cognitive Aging in HIV-Associated Neurocognitive Disorders (HAND)

By

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To my incredibly supportive family: Jonathan, Lystra, Lloyd, Veronica, Kamana, Janaye and Kircil

To my friends near and far, for believing in me at times even more than I did

and

In loving memory of my beloved Mother, Viola

Thank you for everything.

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LIST OF ABBREVIATIONS

ACh: Acetylcholine

AChEI: Acetylcholinesterase Inhibitor

AD: Alzheimer's Disease

AIDS: Acquired Immuno-Deficiency Syndrome

ANI: Asymptomatic Neurocognitive Impairment

ANOVA: Analysis of Variance

ANCOVA: Analysis of Covariance

APOE: Apolipoprotein-E

APP: Amyloid Precursor Protein

Aβ: Amyloid-Beta

ART: Anti-Retroviral Therapy

BAI: Beck Anxiety Inventory

BBB: Blood Brain Barrier

BDI: Beck Depression Inventory

BOLD: Blood-Oxygen Level Dependent signal

CHARTER: CNS HIV Antiretroviral Therapy Effects Research group

CNS: Central Nervous System

COMT: Catechol-o-methyltransferase

CRC: Vanderbilt Clinical Research Center

CRT: Choice Reaction Time Task

CPE: CNS Penetration-Effectiveness Score

CPT: Connors Continuous Performance Task

D1DR: Type 1 Dopamine Receptor

D2DR: Type 2 Dopamine Receptor

DAN: Dorsal Attention Network

DMN: Default Mode Network

DNA: Deoxyribonucleic Acid

DSST: Digit Symbol Substitution Task

ECG/EKG: Electrocardiogram

FCN: Frontoparietal Control Network

fMRI: Functional Magnetic Resonance Imaging

FOA: Focus of Attention

GABA: Gamma-Aminobutyric Acid

GP: Grooved Pegboard Task

gp120/41: Glycoprotein 120/41

HAD: HIV-Associated Dementia

HAND: HIV-Associated Neurocognitive Disorder

HAROLD: Hemispheric Asymmetry Reduction in Older Adults

HGN: Horizontal Gaze Nystagmus

HIV: Human Immunodeficiency Virus

IHDS: International HIV Dementia Scale

mAChR: Muscarinic Acetylcholine Receptor

nAChR: Nicotinic Acetylcholine Receptor

MCI: Mild Cognitive Impairment

MND: Mild Neurocognitive Disorder

MECA: Mecamylamine

MFQ: Memory Functioning Questionnaire

MoCA: Montreal Cognitive Assessment

MRI: Magnetic Resonance Imaging

nAChR: Nicotinic Acetylcholine Receptor

OCL: One-Card Learning Task (COGState Battery Task)

PAM: Positive Allosteric Modulator

PASA: Posterior-Anterior Shift in Aging

PCAT: Posner Cued Attention Task

PD: Parkinson's Disease

PET: Positron Emitted Tomography

POMS: Profile of Mood States

RNA: Ribonucleic Acid

RT: Reaction Time

SCOP: Scopolamine

SMC: Subjective Memory Complaint

SRT: Selective Reminding Task

SSS: Stanford Sleepiness Scale

TSS: Total Sobriety Score

VAS: Visual Analog Scales

VF: Verbal Fluency Task

CHAPTER I

INTRODUCTION

As the human brain ages, the decline in cognitive ability may be secondary to decreased function of the cholinergic system. Decreases in cholinergic system activity are linked to deficits of a number of cognitive domains, particularly attention, memory, executive functioning, and others. While these cognitive deficits may also occur in healthy aging, there is evidence that they may be exaggerated in adults aging with Human Immunodeficiency Virus (HIV) suggesting that HIV related neurodegeneration may impact the cholinergic receptor system as part of the development of HIV-Associated Neurocognitive Disorders (HAND).

Human Immunodeficiency Virus (HIV-1) affects more than 1 million people in the US (Centers for Disease Control and Prevention, 2018). Though it is still a life-threatening diagnosis, many HIV-positive adults have survived the initial epidemic in the early 1980's and continue to live relatively stable, healthy lives with the disease. As the HIV-positive population survives with the disease, management of the added complications of age-related changes to their physical, mental and cognitive health becomes a more significant challenge. As the face of the HIV-positive population changes, so too should the considerations of clinicians who treat them. Older age is associated with cognitive and motor changes as well as the development of brain pathologies. Cognitive functioning is additionally vulnerable to HIV-related damage, particularly if the individual is infected late in life, goes undiagnosed or untreated for some time after exposure, or if they have lived with the disease for more than a decade.

Cognitively, there are numerous changes that occur as individuals age into their 5th and 6th decades of life and beyond, including decreased processing speed decreased reaction time, reduced

working and episodic memory capacity, and deficits in attention (Salthouse 2010, Grady 2012, Harada, Natelson Love et al. 2013). Multiple studies have also noted various changes to neural activation patterns in older individuals, such as a decreased functional connectivity and reduced de-activation of the default mode network (Betzel, Byrge et al. 2014, Song, Birn et al. 2014, Vidal-Pineiro, Valls-Pedret et al. 2014) and a posterior-anterior shift in activation during cognitive tasks (Davis, Dennis et al. 2008).

It has been proposed that one of the major phenotypic changes resulting from HIV infection is an overall acceleration of the aging process (Deeks 2009, Bhatia, Ryscavage et al. 2011, Horvath and Levine 2015), which may include cognitive performance as well. This hypothesis has not been fully supported, as other investigators finding no evidence of an interaction between HIV and the aging process (Ances, Vaida et al. 2010). Though the gross structural changes associated with HAND (including deep fronto-striatal white matter, hippocampus, thalamus, basal ganglia, striatum, sensorimotor neocortex) have been identified (Vance 2004, Schiller, Foley et al. 2009, Kallianpur, Kirk et al. 2011, Kuper, Rabe et al. 2011, Spudich and Ances 2011, Silva, Rodrigues et al. 2012, Ipser, Brown et al. 2015, Watson, Busovaca et al. 2017), there has been little investigation into the neurotransmitter systems that may be particularly vulnerable to HAND related damage as individuals with HIV age. As research into the aging HIV population continues, it is important in this population to study neurotransmitter systems in the brain that have already been associated with the cognitive aging process, such as the cholinergic system. Existing knowledge of age-related changes to the cholinergic system, the cognitive impairment associated with dysfunction, and how they may overlap with cognitive symptoms seen in HIV+ individuals, will provide useful insight into how mechanisms of the cognitive aging process are altered by the presence of HIV, and ultimately if cholinergic treatments may be beneficial.

The combination of pathological processes may interact, resulting in an HIV-positive population that may experience significant impairments earlier than their HIV-negative counterparts. Current

estimates indicate that roughly 50% of the US HIV-positive population is currently aged 50 years or older (Goodkin, Wilkie et al. 2001, Becker, Lopez et al. 2004, Vance, McGuinness et al. 2011, Centers for Disease Control and Prevention, 2018), which makes understanding the unique nuances of aging with HIV a concern for researchers, clinicians, and patients alike. The following chapters will review the cognitive, clinical, and behavioral considerations facing a “greying” HIV-positive population, and factors that may contribute to better outcomes for older adults living with this disease.

CHAPTER II

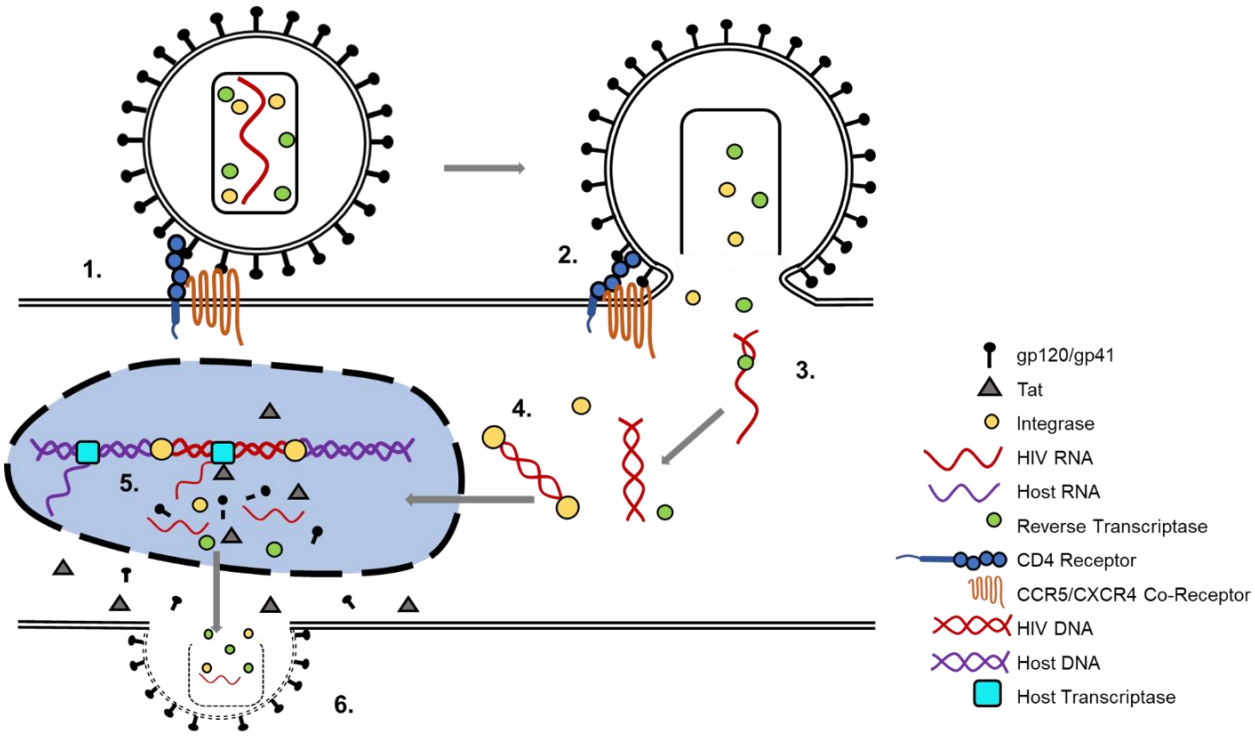
HIV AND THE CENTRAL NERVOUS SYSTEM

Pathology of HIV

The Human Immunodeficiency Virus (HIV), a virus that targets and causes progressive deterioration of human immune cells, was first observed in the US in the early 1980's as an unknown source of a severely depleted immune system, resulting in opportunistic infections. The disease was given the name "HIV" by the late 80's, and is now known to be the cause of Acquired Immuno-Deficiency Syndrome, or AIDS (Rosen 1985, Levy 1993, Weiss 1993). Spread primarily through sexual contact, but also through improper needle hygiene or from mother-to-child, the HIV virus is known as a retrovirus, which contains viral RNA instead of viral DNA (Weiss 1996). The HIV viral capsule contains a number of necessary proteins and enzymes that allow it to take over the functionality of the target host cell (Pavlakis and Felber 1990): reverse transcriptase, which translates HIV-RNA to HIV-DNA (Telesnitsky and Goff 1997, Isel, Ehresmann et al. 2010, Hu and Hughes 2012), integrase, which inserts viral DNA into the host-cell DNA (Shin, Taddeo et al. 1994, Tekeste, Wilkinson et al. 2015); Tat, a protein that facilitates the translation of inserted viral DNA to RNA and subsequently new viral proteins (Marciniak, Calnan et al. 1990, Rosen and Pavlakis 1990), and protease, which cleaves the pre-cursor HIV proteins at key locations to produce mature HIV proteins that will then be assembled into new HIV viral particles (Peng, Ho et al. 1989, Shoeman, Honer et al. 1990). The virus preferentially targets and infects cells that express the CD4 receptor, in addition to either the CCR5 or CXCR4 co-receptors (Deng, Liu et al. 1996, Dragic, Litwin et al. 1996, Bjorndal, Deng et al. 1997, Berger, Murphy et al. 1999), bonding with the viral envelope protein protrusions comprised of two proteins: gp120 and gp41 (McKeating and Willey 1989, Freed, Myers et al. 1990, Eiden and Lifson 1992).

After successful binding of the viral surface proteins to the target cell's receptors, internal cellular mechanisms fuse the viral membrane with the cellular membrane, opening a pore allowing the viral capsule contents to be introduced to the cell interior, and the cellular infection to begin (Chan and Kim 1998), as depicted in Figure 1. Once exposed to the virus, the body attempts to attack the foreign virus, initiating an immune response by increasing delivery of monocytes and lymphocytes to the affected tissues (Clark and Shaw 1993, Henrard, Daar et al. 1995). However, monocytes and lymphocyte cells of the human immune system are "CD4+", meaning they express the target receptors that the HIV virus surface proteins must bind to in order to successfully infect the host cell (Geleziunas, Bour et al. 1994, Law, Satija et al. 2016). As these cells are subsequently infected, re-programmed, and ultimately killed, the body cannot replace them quickly enough to compensate, and this individual is then considered HIV-positive (Schacker, Collier et al. 1996, Rosenberg and Cotton 1997, D'Souza, Axten et al. 2010).

Disease progress is commonly monitored by physicians by monitoring CD4+ cell counts, or the total number of CD4+ cells present, as well as viral load, the number of viral particles present, in a given volume of blood (Wood, Yip et al. 2000, Bisson, Gross et al. 2006). These two numbers tend to move opposite each other, and the goal of treatment is to increase CD4+ counts into normal ranges of 500-1,500 (indicating the presence of a functioning immune system) and decrease viral load to <50 copies (indicating significantly reduced, or undetectable viral proliferation) (Carr, Emery et al. 1996, Mellors, Rinaldo et al. 1996, Smart 1996). Patients with high viral load (10,000 or more) and low CD4+ counts (200 or less) are considered AIDS-positive and are at significantly higher risk for development of life-threatening opportunistic infections (Stein, O'Sullivan et al. 1992, Walsh and Calabrese 1992).



1. HIV Virion binds to host cell CD4 receptor and Co-Receptor, via the viral gp120/gp41 surface protein complex
2. Receptor/Co-Receptor complex is activated, unzipping viral membrane into the host cell as internal capsid dissolves and releases HIV RNA, reverse transcriptase, and integrase proteins
3. HIV Reverse Transcriptase translates viral RNA into DNA
4. New viral DNA molecule is chaperoned into the nucleus with integrase proteins, which insert the viral DNA into the host's DNA sequence
5. Host cell begins to transcribe HIV DNA into new HIV proteins, including Tat, which upregulates the transcription of the HIV sequences vs. the Host sequences
6. Newly assembled viral proteins, enzymes, RNA and other particles begin to form new HIV virions, which bud off the host cell surface

FIGURE 1: Process of HIV-Virus Infection of a CD4+ Cell

Successful HIV treatment inhibits the proliferation of the virus as soon as possible after exposure, to prevent the spread of the virus within the human host and preventing transmission to others (Samji, Cescon et al. 2013). The first line treatment for HIV is administration of one or more of the five classes of antiretroviral therapies (ART), each of which targets a different step in the viral replication cycle: Nucleoside/Nucleotide Reverse Transcriptase Inhibitors are non-functional analogues for cellular nucleosides which bind to the forming HIV-DNA strand in place of real nucleosides, preventing the new viral DNA strand from being completed; Non-Nucleoside/Nucleotide Reverse Transcriptase Inhibitors bind to a nearby allosteric site of reverse transcriptase molecules, preventing

nucleosides from binding correctly to the orthosteric site; Protease Inhibitors block the enzyme protease, which is necessary to cleave viral pre-cursor proteins into mature, functioning viral particles; Integrase Inhibitors inhibit the process of inserting viral DNA into the host cell's DNA; and Fusion Inhibitors inhibit the virus' ability to bind successfully to the CCR5 co-receptor and fuse with the cell membrane to introduce viral contents into the cell (Warnke, Barreto et al. 2007, De Clercq 2013). Because of the rapid replication cycle of the HIV-virus, it is highly susceptible to mutations that can render certain medications less effective (Rong, Feng et al. 2007, Ibe and Sugiura 2011), and to counteract this, physicians may also perform a genetic screening of an individual's virus to test for common mutations and adjust therapeutic regimens accordingly on a case-by-case basis.

NeuroHIV: Infiltration and Effects of Chronic CNS HIV-1 Infection

The HIV virus travels to the brain within the first two weeks after primary exposure and infection, during the acute phase (Davis, Hjelle et al. 1992, An, Groves et al. 1999, Schnell, Price et al. 2010). As with all pathogens in the peripheral blood stream, HIV-infected monocytes and free viral particles in the blood encounter the Blood-Brain Barrier, a system of tight cellular junctions around blood vessels in the brain which prevents most medium- to-large molecules, bacteria, and viruses from diffusing into brain tissue, where they may be able to do so in the periphery (Ballabh, Braun et al. 2004). However, infected peripheral CD4+ monocytes and lymphocytes in the bloodstream can release free viral particles across the BBB, exposing perivascular monocytes and epithelial cells and CD4+ glia to the virus (Ancuta, Kamat et al. 2008). Once in the brain tissue, the virus can directly infect astrocytes and oligodendrocytes, which like monocytes and lymphocytes, are CD4+ (Schnell, Joseph et al. 2011, Law, Satija et al. 2016). These infected neural cells replicate the virus and subsequently die, releasing a host of neurotoxic chemokines including Tnf, Vpr, interleukin 6, nitric oxide, and quinolinic and arachidonic acids (Fiala, Looney et al. 1997, Kuller, Tracy et al. 2008, Neuhaus, Jacobs et al. 2010), as well as unpackaged Tat and gp120 proteins, which have neurotoxic effects even when not part of a completed, mature virion (Gonzalez-Scarano and Martin-Garcia 2005). The resulting inflammatory cascades from

these chemokines trigger the persistent recruitment and activation of microglia, the brain's primary immune cells, which attempt to clear the brain of the dead and dying cells, as well as the viral particles, apoptotic chemokines, and cell debris, causing a persistent inflammatory response within the brain parenchyma (Kaul and Lipton 1999, Zauli, Secchiero et al. 2000, Garden 2002). Together, primary infection and secondary effects of this excessive neuroinflammation leads to the progressive death of neuroprotective glial cells (oligodendrocytes and astrocytes) leaving local neurons vulnerable to glutamate excitotoxicity, apoptosis, resulting in progressively worsening neurodegeneration (Grant 2008, Harezlak, Buchthal et al. 2011).

Once introduced to the brain tissue, HIV-related damage follows a selective "sub-cortical" pattern initially, accelerating deterioration of connectivity, processing, and association areas (Pfefferbaum, Rogosa et al. 2014). Later damage spreads into gray matter and cortical regions, typically affects fronto-striatal structures and both structural and functional connectivity (Melrose, Tinaz et al. 2008, Ipser, Brown et al. 2015) as well as hippocampal tissue (Castelo, Sherman et al. 2006) and motor areas (Itoh, Mehraein et al. 2000, Sullivan, Rosenbloom et al. 2011). Abnormal fMRI pre-frontal activation during working memory tasks (Ernst, Chang et al. 2002), increased measures of inflammation and diffusivity (Chang, Wong et al. 2008), and reduced resting state functional connectivity within the lateral occipital network (Wang, Foryt et al. 2011), and overall cortical thinning (Thompson, Dutton et al. 2005, Kallianpur, Kirk et al. 2011) have all been observed in HIV-positive adults relative to HIV-negative controls, and in many of these studies the structural changes were in excess of that to be expected given participant age.

Treatment of Central Nervous System (CNS) infection by the HIV-virus is also complicated by the presence of the BBB, which prevents many large or potentially toxic molecules from entering the CNS tissue from the peripheral circulation (Ballabh, Braun et al. 2004). However, the ability of the virus to bypass the BBB while many ARTs cannot, turns the central nervous system into a viral "reservoir"

where the virus can continue to replicate without being affected by treatment (Anthony, Ramage et al. 2005, Alexaki, Liu et al. 2008). This allows for 1. the continuation of neuroinflammatory and neurotoxic effects of the virus to progress, even in virally suppressed, well-controlled HIV-positive individuals (Harezlak, Buchthal et al. 2011), 2. The opportunity for the virus to mutate repeatedly, increasing the risk of resistance to ART regimens (Schnell, Price et al. 2010) as well as 3. the potential for viral particles to escape back into the peripheral circulation and seed new increases in viral activity, or "blips", despite medication adherence (Di Mascio, Markowitz et al. 2003, Mukerji, Misra et al. 2017). The time between initial infection and initiation of treatment has been shown to have an effect on extent of CNS damage (Crum-Cianflone, Moore et al. 2013), essentially the less time the virus is able to replicate peripherally, the better cognitive outcomes, and lower risk of developing cognitive impairments.

The success of ART medication in the current era is partially responsible for the growing number of HIV-positive adults thriving into their 50's, 60's, and beyond (Bhatia, Ryscavage et al. 2011, Moore, Fazeli et al. 2014). However, many of these medications have the potential for neurotoxic side-effects, some of which can be equally, if not more toxic than the effects of the virus itself (Decloedt, Rosenkranz et al. 2015, Underwood, Robertson et al. 2015, Shah, Gangwani et al. 2016). Central Penetrance Effectiveness score (CPE) score, a metric developed by researchers in the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) group, ranks different ART drugs from 1-4, for how well it can pass through the blood brain barrier and enter the CNS: CPE scores have been shown to correlate with cognitive outcomes, with many patients experiencing more severe cognitive impairments with high CPE score regimens vs. lower (Letendre, Ellis et al. 2010). Several studies have also shown that in older HIV patients, changing from a high to a lower CPE regimen can recover some cognitive functioning (Bogoch, Davis et al. 2011, Carvalhal, Gill et al. 2016). Overall, it seems that balancing between central penetrance, peripheral efficacy, and time between exposure and initiation of treatment, leads to the most positive neurocognitive outcomes.

HIV-Associated Neurocognitive Disorders

HIV-Associated Neurocognitive Disorders, or HAND is a spectrum of cognitive deficits that are a common outcome of chronic HIV-1 virus infection, affecting more than 50% of seropositive individuals over the course of their life with the disease (Cysique, Maruff et al. 2004, Gisslen, Price et al. 2011, Heaton, Franklin et al. 2011, Chan and Brew 2014). HAND symptoms result from the underlying process of neurodegeneration caused by the infiltration of the HIV virus into the brain and the neuroinflammation it causes, and is characterized by a number of cognitive deficits (Woods, Moore et al. 2009). HAND is identified diagnostically by the "Frascati Criteria" (Antinori, Arendt et al. 2007): neurocognitive performance at least 1 standard deviation below the mean (normalized for age and education) in at least 2 of the following domains of cognition: language, attention, working memory, learning, information processing, sensory perception, and motor skills.

HAND symptoms are categorized by their severity as follows: The mildest form, asymptomatic neurocognitive impairment (ANI), involves an objective cognitive deficit, with no objective effect on daily functioning; the moderate form, mild neurocognitive disorder (MND), involves objective cognitive deficit, as well as subjective impairment in daily functioning; and the most severe form, which is also the least prevalent form, is HIV-associated dementia (HAD), which is rarely seen except for in severely immunocompromised, end-stage AIDS patients . Symptoms tend to progress from ANI to MND with longer infection time, though early ART medication has reduced prevalence of HAD (Baldewicz, Leserman et al. 2004). There is evidence that patients may also go back and forth between ANI and MND over the course of their disease, which may be attributed to changes in the virus itself due to mutations, changes in medication adherence, effectiveness, or tolerance, or simply due to aging and changes in lifestyle (Lojek and Bornstein 2005, Rickabaugh, Kilpatrick et al. 2011).

HAND Subtype	Criteria
Asymptomatic Neurocognitive Impairment (ANI)	• At least two (2) impaired domains of cognitive performance • Cognitive performance at least one (1) standard deviation below age/education matched means
Mild Neurocognitive Disorder (MND)	• At least two (2) impaired domains of cognitive performance • Cognitive performance at least one (1) standard deviation below age/education matched means • Mild-to-moderate impairment in daily functioning • No evidence of delirium or dementia
HIV-Associated Dementia (HAD)	• At least two (2) impaired domains of cognitive performance • Cognitive performance at least two (2) standard deviations below age/education matched means • Moderate-to-severe impairment in daily functioning • No evidence of alternative causes for dementia

TABLE 1: Characteristics of HIV-Associated Neurocognitive Disorder Subtypes

HAND severity over time can be predicted by a CD4 nadir, the first CD4+ cell count after a confirmed HIV-positive diagnosis (Ellis, Badiee et al. 2011). As it has been shown that HIV can migrate into the brain tissue within the first 1-2 weeks after initial exposure, reducing the time between exposure and treatment initiation has been shown to improve cognitive outcomes later in life (Lok and DeGruttola 2012, Medland, Chow et al. 2017) The impaired immune response caused by HIV leads to a premature, persistent inflammatory state(Kuller, Tracy et al. 2008, Deeks, Tracy et al. 2013). When this is combined with the neurological and cognitive changes associated with aging, presumably physiological and “cognitive weathering” may occur more rapidly (Le Saux, Weyand et al. 2012).

There have been numerous studies to-date exploring the effects of the HIV virus on brain and cognitive functioning in otherwise well-suppressed and medically adherent individuals. It has been shown that cognitively asymptomatic HIV+ individuals displayed abnormal visual spatial attention processing compared to seronegative controls (Maruff, Malone et al. 1995), another found that HIV+ individuals showed significant deficits in verbal working memory storage and processing (York, Franks et al. 2001), and another group showed immediate, delayed (auditory/visual), and working memory deficits in HIV+ males compared to controls, which they attributed to subcortical gray and white matter

damage due to HIV infection (Schiller, Foley et al. 2009). There has also been investigation into biomarkers for cognitive outcomes in adults with HIV.

The Aging Face of the HIV-Positive Population

Less is known about the cognitive consequences of aging with HIV compared to normal aging, due to the fact that many survivors of the HIV-positive community are only now entering late-life. One study found that in HIV-positive individuals, age over 50 years increased the risk of developing a cognitive disorder three-fold compared to HIV-negative controls (Becker, Lopez et al. 2004), and another found that aging and positive HIV status had an adverse, additive impact on learning and executive functioning (Iudicello, Woods et al. 2012). In contrast, large cohort studies evaluating the effects age and HIV status on neuropsychological performance, found no evidence for increased impairment of performance (Cysique, Maruff et al. 2011, Valcour, Paul et al. 2011).

Because of advances in ART medication, the milder cognitive impairments of HAND (ANI and MND) are becoming more common, and HAD is becoming less common (Ances and Ellis 2007), so understanding the mechanisms of these impairments as well as any interaction with age has implications for long-term treatment and quality of life as the HIV-positive population lives longer. As research into the aging HIV population continues, it may be useful in this population to study cell and neurotransmitter receptor populations in the brain that have already been associated with the cognitive aging process, such as the cholinergic receptor system. Existing knowledge of age-related changes to these systems, the cognitive impairment associated with dysfunction, and how they may overlap with cognitive symptoms seen in HIV-positive individuals, could provide useful insight into how mechanisms of the cognitive aging process are altered by the presence of HIV, and how neurotransmitter-based treatments may be beneficial.

There is also evidence that in HIV-positive adults, risk biomarkers related to Alzheimer's disease confer an additional burden. HIV-positive adults with at least one APOE-e4 gene have been found to have more severe markers of brain atrophy (Wendelken, Jahanshad et al. 2016), higher risk of developing the most severe form of HAND (Valcour, Shikuma et al. 2004), and higher deposition of damaging brain amyloid (Green, Masliah et al. 2005). One study found that in HIV+ individuals, age over 50 years increased the risk of developing a cognitive disorder three-fold compared to HIV- controls (Becker, Lopez et al. 2004), and another found that aging and positive HIV status had an adverse, additive impact on learning and executive functioning (Iudicello, Woods et al. 2012). In contrast, large cohort studies evaluating the effects age and HIV status on neuropsychological performance, found no evidence of increased impairment of performance (Cysique, Maruff et al. 2011, Valcour, Paul et al. 2011).

Aging with HIV: Accelerated or Accentuated?

There is controversy about whether the relationship between aging and HIV disease is additive or interactive (Deeks 2009, Bhatia, Ryscavage et al. 2011, Cysique, Maruff et al. 2011, Iudicello, Woods et al. 2012). Though it has been shown that individuals with HIV may indeed be at higher risk for cognitive decline (Becker, Lopez et al. 2004) and there is evidence that the presence of the virus in the body can contribute to acceleration of the aging process (Le Saux, Weyand et al. 2012), there is controversy regarding whether the two processes interact. There are two possible models for how this may work: either in the presence of the virus cognition is impaired, but performance continues to decline with advancing age at the same rate as those without HIV (with no interaction with aging) (Figure 1A) or the presence of the virus may indeed interact with the progression of age related cognitive decline, and produce a steeper slope of decline in cognitive status compared to the aging process in those without HIV (Figure 1B). The acceleration of cognitive decline in the second model, may indicate that HIV-positive status aggravates the loss of cholinergic and other neurotransmitter

system functioning that is associated with cognitive aging, and understanding if these two processes interact has important implications for understanding the aging process in HIV.

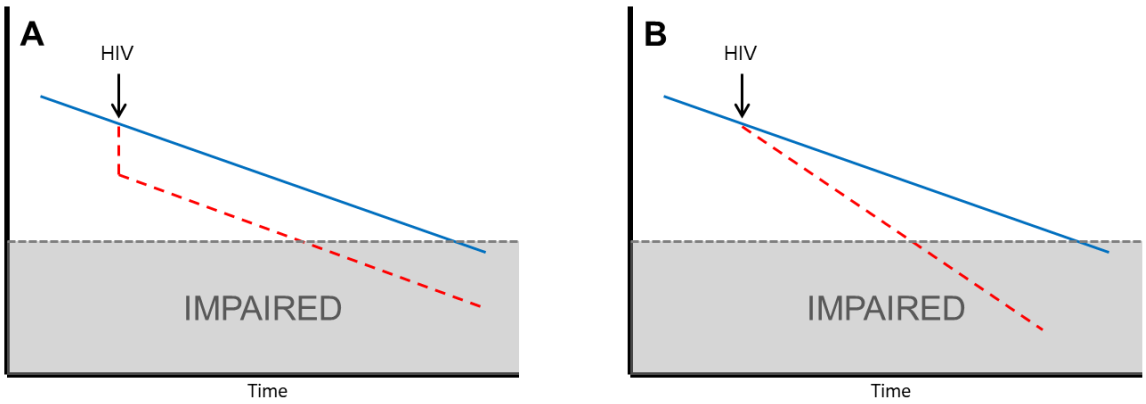


FIGURE 2: Hypothesized Models for Progression of Cognitive Decline with HIV

The literature is divided between these two models, and the presence or absence of the interaction between age-effects and HIV-effects may rely on a number of factors, such as the size of the sample, the design of the study and the measures employed, the overall age of the sample, as well as biological factors like CD4 nadir (which has been shown to be a robust predictor of cognitive status later in life (Ellis, Badiee et al. 2011)), ART combination, viral load, and other lifestyle factors. In order to understand which of these models is more accurate, examining the functioning of the cholinergic receptor system in HIV and comparing relative responses to antagonist medications will provide insight into whether the process of age-related cognitive decline and HIV may be affecting similar cognitive domains and/or brain systems that may change with aging.

CHAPTER III

NEUROBIOLOGICAL AND COGNITIVE CHANGES OF THE AGING BRAIN

Starting in the 4th and 5th decades of life, the human brain begins to undergo numerous changes to maintain cognitive, executive and memory functioning, as it faces neural atrophy, changes in connectivity, and slowing and general fatigue of the biological processes that underlie normal healthy brain performance. Collectively, these changes can be defined by the term “Cognitive Aging”, or the progressive deterioration of mental functioning overtime due to advanced age (Bartus, Dean III et al. 1982).

Impaired Domains of Cognitive Aging

To better understand cognitive aging, it is important to consider what domains of cognition are vulnerable, or resistant, to age-related impairment. Most commonly affected domains of cognition in the aging brain are attention, memory (episodic and working), psychomotor processing speed, and executive function, and each through slightly differing neurobiological mechanisms.

Attention

The domain of attention is heavily controlled by nicotinic cholinergic signaling in normal and aging brains, which is rapidly up-regulated in response to new, salient information in a healthy younger brain, but becomes dysregulated in older or impaired individuals (Burk, Herzog et al. 2002, Sarter, Hasselmo et al. 2005, Thienel, Voss et al. 2009). Nicotinic stimulation can improve signal-to-noise ratio within this domain, as well as enable more fluid switching between stimuli, which may in turn improve other markers of cognitive performance (reaction time, discrimination, etc.) (Weissman, Roberts et al. 2006, Hahn, Ross et al. 2007, Asgaard, Gilbert et al. 2010). The cholinergic receptor system has been shown to be involved in deactivating the Default Mode Network (DMN) (Vidal-Pineiro, Valls-Pedret et al.

2014, Li, Motelow et al. 2015) which is normally most active during passive rest, and instead increasing activity in task-relevant networks such as the Dorsal Attention network (DAN) or Frontoparietal Control network (FCN) to improve performance (Grady, Sarraf et al. 2016)

Memory

The human memory system, mostly centered in the functioning of the bilateral hippocampal formation (Scheibel 1979, Thompson and Kim 1996, Schiltz, Szentkuti et al. 2006) is the process of encoding and storing new information into reinforced, repeated neural pathways for subsequent retrieval. Minor to moderate lapses in memory functioning are a common complaint for older individuals (Bartley, Bokde et al. 2012, Harada, Natelson Love et al. 2013, Ronnlund, Sundstrom et al. 2015), and is associated with reduction in hippocampal volume (Schiltz, Szentkuti et al. 2006). Age-related memory failures have been associated with dysfunctional signaling pathways between medial temporal lobe structures and the rest of the brain (Ward, Mormino et al. 2015). The hippocampus is particularly enriched with muscarinic and nicotinic cholinergic receptors (Pohorecki and Domino 1987, De Lacalle, Lim et al. 1994, Court, Lloyd et al. 1997) and the loss of receptors as well as the atrophy of the cholinergic nuclei that project there are particularly involved in the symptoms of impaired memory function with older age. Memory functioning in older and elderly persons can be further complicated by the additional diagnosis of geriatric depression, (Nebes, Butters et al. 2000, Crane, Bogner et al. 2007, Dumas and Newhouse 2015) shown to further diminish hippocampal activity compared to non-depressed adults.

Psychomotor Speed

Psychomotor slowing, or the diminished speed from stimuli, to cognitive processing, to initiation of a motor response, is commonly seen in advanced age (Hicks and Birren 1970, Falkenstein, Yordanova et al. 2006). Compared to younger healthy adults, the identification, processing, and response time to exogenous stimuli gradually slows over the lifespan, both cognitively and motorically

(Salthouse and Coon 1993, Salthouse 1994, Rodriguez-Aranda, Waterloo et al. 2006). Several regions of the brain are involved in this process, from top-down attention and executive control in the frontal and pre-frontal cortices, signal processing in the parietal and cingulate cortices, and subcortical regions like the basal ganglia, and thalamus, for motor preparation (Hicks and Birren 1970, Falkenstein, Yordanova et al. 2006, Solbakk, Fuhrmann Alpert et al. 2008), as well as the necessary white matter tracts that connect them (Borghesani, Madhyastha et al. 2013). As these gray and white matter regions atrophy due to age, the indirect secondary pathways require more time to produce the desired response, leading to overall slowing (Falkenstein, Yordanova et al. 2006).

Executive Control

Finely tuned executive control is one of the primary functions of the frontal and pre-frontal cortex (Fassbender, Murphy et al. 2004, Taylor, Welsh et al. 2004) by integrating novel stimuli, past experiences, and critical thought processes to influence and monitor behavior (Hunt and Kingstone 2004). Behavioral inhibition is a major function of this system, and changes to inhibitory activity has been shown in older healthy adults as well as those in the early stages of mild cognitive impairment (MCI) and Alzheimer's disease (AD) (Sebastian, Baldermann et al. 2013, Anderson, Healey et al. 2016, Kleerekooper, van Rooij et al. 2016). The ability to discriminate between salient and distracting information and inputs is also a function of the executive control system, (Mott, Alperin et al. 2015). In older individuals, this ability is diminished, and has been shown to affect many other domains of cognitive functioning, such as attention (Basak and Verhaeghen 2011), motivation, and goal-directed decision processing (Hollerman, Tremblay et al. 2000, Langley, Fuentes et al. 2001, Taylor, Welsh et al. 2004), and memory (Goh, An et al. 2012), as connectivity between the frontal lobes and the rest of the brain are lost. The executive control network (Connolly, McNulty et al. 2016) one of the large-scale brain networks identified in the human brain, has been shown to become less efficient with age, as these regions are otherwise recruited in a compensatory manner to maintain cognitive performance in other domains (Traykov, Raoux et al. 2007, Wu, Soder et al. 2014) and subsequently less of these

areas continue to be solely dedicated to executive processing (Geerligs, Maurits et al. 2014, La, Mossahebi et al. 2015, Saverino, Fatima et al. 2016).

As the human brain ages, though there may be subtle differences in time scale or severity, the overall mechanism at work is de-differentiation of neural tissues, as the clear distinctions between discrete networks, regions, and domains become blurred, a result of receptor, white matter, connectivity, and gray matter loss of function. In an effort to re-wire and compensate for these losses, the brain overall continues to function sub-optimally, until those deficits can no longer be overcome, resulting in the collection of impairments we have come to associate with old age. These global and small-scale changes are impactful even in a healthy brain, and understanding how those changes take place in a non-"healthy" brain is a vital next step in further understanding the intersection between age and disease.

Neurotransmission and Connectivity Alterations with Age

The primary mechanism that affects neurobiological functioning with age is gradual decrease in function of necessary receptors in key cell populations: as synaptic strength is lost, remaining receptors must cover the deficit, and when they cannot, noticeable symptoms of aging emerges. Neurotransmitter systems including the dopamine, glutamate, and acetylcholine systems lose nuclei and projections (Freeman and Gibson 1988, Schroder, Giacobini et al. 1991, Nordberg, Alafuzoff et al. 1992), and are also involved in some of the secondary effects of aging such as memory dysfunction, physical slowing, and mood disorders, as well as pathological changes such as Alzheimer's disease, Parkinson's disease, geriatric depression, and other dementing disorders (Rathmann and Conner 1984, Kalayam and Alexopoulos 1999). Overall gray matter is lost with progressing age, starting as early as the 30s (Saykin, Wishart et al. 2006, Montembeault, Joubert et al. 2012). Much of the earliest gray matter loss occurs in the bi-lateral medial temporal lobes, affecting the hippocampus (Decker 1987, Pruessner,

Collins et al. 2001, Steffens, McQuoid et al. 2011), amygdala (Zanchi, Giannakopoulos et al. 2017), paralimbic (Grieve, Clark et al. 2005, Driscoll, Davatzikos et al. 2009, Salat, Chen et al. 2011), and basal ganglia (Vivo, de Vera et al. 2001, Rosano, Bennett et al. 2012).

The presence of catecholamine neurotransmitters, specifically dopamine, is necessary for normal functioning of complex neurological and cognitive functioning (Nieoullon 2002). Originating in the basal ganglia (nucleus accumbens, ventral tegmental area, substantia nigra, striatum) and heavily enervating the frontal cortices, dopamine availability is involved in attention, decision making, learning and reward processing (Vitiello, Martin et al. 1997). In the context of cognition, dopamine activity is important for reward and decision processing (Hollerman, Tremblay et al. 2000, Vink, Kleerekooper et al. 2015), visuospatial working memory (Muller, von Cramon et al. 1998), as well as attention and working memory, with modulation from the cholinergic system as well (Levin and Rose 1995, Nieoullon 2002).

Dopamine acts through two main receptor subtypes, the D1DR and D2DR, and the two receptors modulate slightly different cognitive functions (Bolton, Marioni et al. 2010, Stelzel, Fiebach et al. 2013, Tsang, Fullard et al. 2015). Dopamine activity at D2 receptors is associated with memory function, and loss of these receptors with age has been associated with poorer memory functional connectivity between the hippocampus and caudate (Nyberg, Karalija et al. 2016) The release of dopamine is closely modulated by cholinergic receptors, allowing for fine tuning of working memory (Levin and Rose 1995).

Aging has demonstrable effects on the ability of dopamine to regulate cognitive functions, particularly as receptors and dopaminergic cells are lost in cases of pathological aging. Older age is associated with lower dopaminergic activity in the ventral striatum and pre-frontal cortex, resulting in poorer ability to process reward (Vink, Kleerekooper et al. 2015). Dopamine has also been shown to be

involved in the process of memory dedifferentiation (the loss of distinct neural representations of information), and reduced dopamine availability is associated with poorer memory specificity in older adults (Abdulrahman, Fletcher et al. 2015). Older adults also show decreases in dopamine mediated measures of cognitive flexibility, Loss of D1DR density in the caudate and dorsolateral pre-frontal cortex in older adults is associated with poorer working memory performance and higher blood-oxygen level dependent (BOLD) neural activation variability (a measure of lower task attention) (Guitart-Masip, Salami et al. 2016). Decreased connectivity has been found between the locus coeruleus (another dopaminergic region), with parahippocampal cortex in older adults (Zhang, Hu et al. 2016), consistent with other studies that have linked this loss of connectivity to working memory, attention and executive control impairments of mild cognitive impairment. Genetically, polymorphisms in the catechol-o-methyltransferase (COMT) gene, necessary for dopamine metabolism, also have distinct effects on stability of cognitive functions with age (Papenberg, Backman et al. 2014). The Val158Met polymorphism has been assessed in studies of dopamine availability and the subsequent impact on cognitive ability: one study found that older adults homozygous for the COMT*Val allele performed significantly more poorly on both working memory and episodic memory tasks compared to heterozygous or homozygous COMT*Met adults (Papenberg, Backman et al. 2014), and significant associations with decreased cortical thickness in older adults have also been identified (Starr, Fox et al. 2007, Lee and Qiu 2016)

As nuclei producing these neurotransmitters, as well as their cortical and sub cortical targets are lost as a result of pathological conditions of cognitive aging, several studies have shown changes in cell biomarkers that underlie these dysfunctional NT systems. Blockade studies were an early tool to explore the effects of loss of function to these systems, and the effects of these can mimic some of the commonly expected signs of aging (Vitiello, Martin et al. 1997). Progressive loss of dopamine producing neurons in the substantia nigra (Anglade, Vyas et al. 1997) and decreasing binding to the dopamine transporter in the striatum and basal ganglia (Erixon-Lindroth, Farde et al. 2005) have been

shown in Parkinson's, and are associated with poorer reward processing, episodic memory function, and may also lead to motor dysregulation and early symptoms of this pathologic aging disorder (Volkow, Gur et al. 1998, Chau, Roth et al. 2004, Guitart-Masip, Salami et al. 2016, Nyberg, Karalija et al. 2016). Task-related BOLD signaling has also been shown to be altered in the brains of older vs. younger adults (Guitart-Masip, Salami et al. 2016), resulting in both higher off-task activity and lower on-task activity, as well as slower responses, overall negatively impacting performance in the older adults. Decreased brain volume and dopamine signaling in the fronto-temporal cortices is also associated with mood disturbances in older adults, contributing to increasing symptoms of depression (Taylor, MacFall et al. 2005, Dotson, Davatzikos et al. 2009).

Cognitive aging may also be partly due to a decline in cholinergic system function and the progressive loss of cholinergic projections from the basal forebrain nuclei (Schliebs and Arendt 2011). Decrease in projections, and decrease in expression of nicotinic and muscarinic acetylcholinesterase receptors (nAChRs and mAChRs) with age results in an increase in sensitivity to cholinergic blockade (Sunderland, Tariot et al. 1988). In particular, impairment of the nAChR system is associated with deficits of attention and working memory, and thought to be involved in the cognitive symptoms seen in Alzheimer's disease, Parkinson's disease, and others, such as slowed reaction time, sustained attention deficits, sensory gating and working memory problems (Mihailescu and Drucker-Colin 2000). It has been shown that availability of nAChRs decreases with age, and this loss is more profound in individuals suffering from diagnosed cognitive disorders (Guan, Zhang et al. 2000, Picciotto and Zoli 2002, Mitsis, Cosgrove et al. 2009, Kendziorra, Wolf et al. 2011, Okada, Ouchi et al. 2013)

Neuroconnectivity, or the degree to which certain areas of the cortex and sub-cortex are linked and function in unison, is another metric that is altered in the brains of older individuals. As cell populations lose synaptic function, existing interconnected networks begin to fail, and the remaining neurons must re-wire to maintain overall functionality of the processes they transduce. The Default

Mode Network (DMN) is one such system vulnerable to the effects of age, normally active at rest, during non-task-related activities (Sambataro, Murty et al. 2010, Horn, Ostwald et al. 2014). Comprised of the anterior and posterior cingulate, medial pre-frontal cortex, medial temporal lobes (including entorhinal cortices and hippocampi) and the inferior parietal (Laird, Eickhoff et al. 2009), and must be deactivated to initiate a task (Anticevic, Cole et al. 2012), in favor of other task-relevant processes and networks to function. The ability of the brain to facilitate this switching from off- to on-task activity in later life is a clear deficit in older adults (Song, Birn et al. 2014, Ng, Lo et al. 2016), and has deleterious effects on attention, working memory, executive control, and processing speed. These effects are caused by the process of dedifferentiation, a consequence of neural re-wiring correlated with age-related functional decline (Vidal-Pineiro, Valls-Pedret et al. 2014): as secondary connections are made, the distinct activity patterns between the various networks become blurred, as the networks become more globally connected between networks and less locally connected within networks (Betzel, Byrge et al. 2014). There is evidence that not only is local vs global connectivity affected, but the efficiency of those new connections is lower as well, requiring more indirect connections to compensate for the original configuration as individuals age(Geerligs, Maurits et al. 2014). In particular, it has been shown that frontal lobe and anterior parietal activation is considerably increased in older adults, however without an appreciable difference in task performance, a phenomenon called the Posterior-Anterior Shift in Aging (PASA) (Davis, Dennis et al. 2008).

Markers of Pathological Cognitive Aging

In addition to age-related atrophy of gray matter, several other biological features are not only associated with aging, but may be exacerbated in pathological aging conditions such as mild cognitive impairment, Alzheimer's disease, Parkinson's disease, and other neurocognitive disorders. The allelic expression of the apolipoprotein-E (APOE) gene also has considerable effects on not just cognitive outcomes over the lifespan. This gene is expressed in 3 variants, E2, E3, or E4 (Wardell, Suckling et al. 1982). The high-risk allele, E4, confers a significant risk for early age-related cognitive impairments,

mild cognitive impairment, and ultimately, Alzheimer's dementia (Fleisher, Sowell et al. 2007, Sutphen, Jasielec et al. 2015), whereas the E2 variation, the least common, has some neuroprotective effects on cognition in older adults (Corder, Saunders et al. 1994). The expression of this gene has effects on many different factors of brain and cognitive health, including abnormal cleavage of amyloid precursor protein (APP) into toxic amyloid-beta (Haass and Selkoe 2007, Resnick, Sojkova et al. 2010, Sojkova, Zhou et al. 2011), increased risk of cognitive impairment and cerebrovascular damage, as well as decrease neurite growth and hippocampal volume (Hull, Strauss et al. 1996, Mawuenyega, Sigurdson et al. 2010, Landau, Mintun et al. 2012, Sagare, Bell et al. 2012).

Preferential cleavage of the amyloid precursor protein into the neurotoxic fragment amyloid-beta 42 (Aβ-42) (Zhao, Long et al. 2012) leads to excessive accumulation and extracellular deposition of the protein fragments, (Geula, Wu et al. 1998), and may overwhelm the brain's ability to metabolize and clear them (Mawuenyega, Sigurdson et al. 2010, Sagare, Bell et al. 2012), which leads to accumulation of amyloid that can trigger apoptosis (Haass and Selkoe 2007). This accumulation and cell loss have been closely associated with increasing risk for cognitive impairment, and development of Alzheimer's disease(Hull, Strauss et al. 1996, Younkin 1998, Okada, Ouchi et al. 2013). Deposition of amyloid worsens with age, and may correlate with the loss of cognitive function (Resnick, Sojkova et al. 2010, Sojkova, Zhou et al. 2011, Landau, Mintun et al. 2012, Donohue, Sperling et al. 2017). Intracellularly, mis-folded microtubule fragments can also assemble into Tau protein tangles, which additionally can have neurotoxic effects when phosphorylated (Rapoport, Dawson et al. 2002, Blair, Nordhues et al. 2013). As various cell types continue to be damaged and die, this also triggers an immune response in the brain, in an attempt to clear potentially dangerous intracellular and extracellular debris. Unfortunately, on-going, persistent microglial activation and inflammatory responses have deleterious effects, which may be exacerbated in those older adults whose brains are already vulnerable due to concurrent patterns of neurodegeneration, neurotoxicity, debris accumulation, and accumulating brain pathology.

Neuroimaging, such as positron-emission tomography (PET) and magnetic resonance imaging (MRI) have identified several features of brain changes that can be observed often before clinical symptoms emerge (Kantarci, Senjem et al. 2011, Betzel, Byrge et al. 2014, Song, Birn et al. 2014) and also can provide insight to the extent of age- and disease- related changes as they progress. The development of new PET ligands that can identify tau (Betthauser, Lao et al. 2017, Smith, Wibom et al. 2018)and amyloid (Choi, Schneider et al. 2012, Akhtar, Xie et al. 2016, Boccardi, Altomare et al. 2016) deposition density within the brain are useful markers of probable neurodegeneration, which may lead to subsequent cognitive symptoms (Resnick, Sojkova et al. 2010, Landau, Mintun et al. 2012, Donohue, Sperling et al. 2017). Volumetric changes overtime in overall cortex can be seen in the shrinking of gray matter from the skull, particularly in the frontal and temporal lobes (Salat, Kaye et al. 1999, Ge, Grossman et al. 2002, Gennatas, Avants et al. 2017), and the enlarging of ventricles (Walhovd, Westlye et al. 2011, Madsen, Gutman et al. 2015). T2-Weighted MRI studies have also been used to identify periventricular white matter hyperintensities (Smith, Johnson et al. 2016, Raina, Zhao et al. 2017, Sudre, Cardoso et al. 2017, Wiseman, Booth et al. 2018) as well as the deterioration of large white matter tracts including the corpus callosum (Weis, Kimbacher et al. 1993, Ota, Obata et al. 2006, Xu, Lu et al. 2011) and frontostriatal fasciculus (Han, Arfanakis et al. 2018).

CHAPTER IV

THE NEURONAL ACETYLCHOLINERGIC SYSTEM AND RELEVANCE TO COGNITIVE AGING

Muscarinic and Nicotinic Acetylcholine Receptors

The human brain features two primary subtypes of receptors that respond to acetylcholine, the metabotropic muscarinic receptor (mAChR) and the ionic nicotinic receptors (nAChR) (Birdsall, Burgen et al. 1976, Caulfield, Straughan et al. 1982, Schwartz and Kellar 1983, Gotti, Fornasari et al. 1997). Though they both can bind acetylcholine, they are named for their preferred alternative binding molecule, muscarine and nicotine (Birdsall, Burgen et al. 1978, Lukas 1989). These two receptor subsystems work together to modulate the activity of various other neurotransmitter systems, such as dopamine, and serotonin (Ellis, Ellis et al. 2006), by acting at axon terminals to increase or decrease the release and availability of these other transmitters, based on finely-tuned activity from the major cholinergic nuclei, the nucleus basalis of Meynert, medial septal nucleus, substantia inominata, and diagonal band of Broca (Mesulam, Mufson et al. 1983, Kellar, Whitehouse et al. 1987, Mesulam and Geula 1988, Perry, Smith et al. 1989). There are three subtypes of cholinergic receptors that have been most closely associated with cognitive ability and subsequently, cognitive deficits, associated with aging: the two primary CNS nAChRs: the α4β2 and α7 subtypes, and the M1 mAChR.

Nicotinic Receptors (α4β2, α7)

Neuronal nicotinic receptors are heteromeric or homomeric ion channels, composed of a combination of 5 α, β, δ, or γ subunits (Gotti and Clementi 2004). The primary two subtypes found in the human brain are the heteromeric α4β2*, and the homomeric α7 receptor: the α4β2 receptor can be comprised of 3-α_4 and 2-β_2 subunits or 2-α_4 and 3-β_2 subunits while the α7 receptor is comprised of 5 α_7 subunits (Jensen, Frolund et al. 2005, Harpsøe, Ahring et al. 2011). In addition to being present on the post-synaptic dendrites of neurons and interneurons (Alkondon, Pereira et al. 2000), nicotinic receptors

are also located on the presynaptic axon terminals of cells that release other major neurotransmitters, such as dopamine, serotonin, glutamate, GABA, or acetylcholine allowing for activation/deactivation of these receptors to up- or down- regulate the release of other neurotransmitters, facilitating more precise synaptic signaling (Levin, McClernon et al. 2006, McKay, Placzek et al. 2007).

Nicotinic receptors, regardless of their subunit composition, are ionotropic, allowing for the movement of sodium (Na+) and/or calcium (Ca^{2+}) ions through a membrane channel when they are activated and open (Gotti, Fornasari et al. 1997, Araki, Suemaru et al. 2002). These two receptor types also differ in the number of orthosteric binding sites available: α7 nAChR have five binding sites versus the α4β2's two, and all binding sites must be occupied for a nAChR to stabilize the 'open' confirmation of the receptor and allow ion flux (Itier and Bertrand 2001).

Muscarinic Receptor (M1/M4)

The family of muscarinic acetylcholine receptors has been classified into five subtypes named M1-5, though M1 and M4 are the most prevalent subtype found in the human brain (Jiang, Li et al. 2014). Muscarinic receptors are G-protein coupled metabotropic receptors, triggering a multi-step intracellular cascade of reactions to transduce the initial signal passed from the previous cell (Birdsall, Burgen et al. 1976, Burgard and Sarvey 1990). This cascade differs slightly between the subtypes of receptors, however in the neuronal subtypes, M1/M4, when the orthosteric binding site is occupied, the receptor changes conformation, activating the Gq protein subunit (Tateyama and Kubo 2013)This conformational change triggers activation of phospholipase C (PLC), producing the secondary molecules IP3 and PIP2, which subsequently activate protein kinase C (PKC) (Thomas, Mistry et al. 2008) Finally, PKC interacts with available Ca^{2+} ions, and can trigger multiple activities within the cell, including triggering metabolic or translational processes downstream of the initial cascade.

Muscarinic and nicotinic receptors are widely spread throughout the cortex to enable them to facilitate wide-spread, pre- and post-synaptic cortical modulation, though there are somewhat higher concentrations in several structures of the medial temporal lobes, as well as frontal lobes where they modulate high-order executive control functions (Perry, Smith et al. 1989, Court, Lloyd et al. 1997, Graef, Schonknecht et al. 2011). The widespread enervation makes the cholinergic system integral for normal functioning, but also very vulnerable to damage as the brain ages, as has been shown by investigating biomarkers, imaging, connectivity, and cognitive markers of aging. These two receptors are each associated with overlapping, but slightly different domains of cognitive performance, with nicotinic receptors associated with attention, executive control, and working memory, and muscarinic receptors being more closely associated with psychomotor functioning, episodic memory, and cognitive processing speed (Greenwood, Lin et al. 2009, Voss, Thienel et al. 2010).

Both of these receptor types are necessary for normal cognitive function, and loss or inactivity of these cholinergic receptors is thought to be one of the primary mechanisms associated with cognitive deficits of aging, as well as the secondary cognitive deficits associated with numerous neuropathological conditions such as schizophrenia, depression, AD, PD, and more (Araki, Suemaru et al. 2002, Chau, Roth et al. 2004). Number of functioning muscarinic receptors decrease with age (White, Hiley et al. 1977), and there is also evidence that the coupling mechanism at the membrane that is necessary to initiate intercellular cascades to transmit incoming signals is impaired, (Janickova, Rudajev et al. 2013). Molecular studies have suggested that the ability of the receptors to subsequently unbind a ligand after successfully transmitting a post-synaptic signal may also play a role, as a receptor which cannot reset for re-activation and prepare to bind to a new ligand will inhibit efficient functioning of the synapse (Rang, Dale et al. 2011).

Cholinergic Theory of Cognitive Aging

Cognitive abilities such as attention and executive control rely on adequate functioning of projections of the acetylcholinergic neurotransmitter system (Bartus, Dean et al. 1982, Warburton and Rusted 1993), which modulate the activity of other neurotransmitter systems via nicotinic and muscarinic acetylcholinergic receptors (Ellis, Ellis et al. 2006). The importance of the cholinergic system to cognition was first understood using temporary blockade studies; cholinergic antagonist drugs such as mecamylamine or scopolamine result in deficits of learning, memory, psychomotor speed, and attention (Birdsall, Burgen et al. 1976, Richardson, Miller et al. 1985, Sunderland, Tariot et al. 1987, Newhouse, Sunderland et al. 1988, Sunderland, Tariot et al. 1988). This effect is more pronounced in older individuals, and more so in those with mild cognitive impairment (MCI)/ Alzheimer's Disease (AD) or other dementing disorders. This increase in sensitivity to blockade with age/pathology may indicate fewer available cholinergic receptors and/or projections (Voss, Thienel et al. 2010).

The similarity between symptoms of cholinergic blockade and the cognitive impairments seen with advancing age are described by the cholinergic theory of cognitive aging (Bartus, Dean et al. 1982), which suggests that the progressive decline of cholinergic receptor activity has a negative impact on cognition, which may be partially rescued by cholinergic agonists. Cognitive aging is partly due to a decline in cholinergic system function and the progressive loss of cholinergic projections from the basal forebrain nuclei (Schliebs and Arendt 2011). Decrease in projections, and decrease in expression of nicotinic and muscarinic acetylcholine receptors (nAChRs and mAChRs) with age results in an increase in sensitivity to cholinergic blockade (Newhouse, Sunderland et al. 1988, Sunderland, Tariot et al. 1988, Newhouse, Potter et al. 1992, Levin 1996). In particular, impairment of the nAChR system is associated with deficits of attention and working memory, and thought to be involved in the cognitive symptoms seen in Alzheimer's disease, Parkinson's disease, and others, such as slowed reaction time, sustained attention deficits, sensory gating and working memory problems (Mihailescu and Drucker-Colin 2000).

Compensatory Functioning of The Cholinergic Receptor System

As the human brain ages, progressive tissue loss occurs in even healthy brains, starting in deep subcortical regions such as the striatum, basal forebrain, thalamus, entorhinal cortex, and proceeds outwards (Salat, Kaye et al. 1999, Ge, Grossman et al. 2002, Driscoll, Davatzikos et al. 2009, Madsen, Gutman et al. 2015). Progressive decrease in projections and expression of nicotinic and muscarinic receptors (nAChRs and mAChRs) with age results in an increase in sensitivity to cholinergic blockade (Sunderland, Tariot et al. 1988) The cholinergic system is believed to function as a compensatory system, increasing output of acetylcholinergic modulatory activity to adjust for losses and deficits in the other neurotransmitter systems as they also lose function, often masking the presence of noticeable cognitive symptoms until later in the process (Nordberg, Larsson et al. 1983, Dumas and Newhouse 2011, Bott, Heraud et al. 2016), as shown in Figure 3, (adapted from (Dumas and Newhouse 2011)) However once the age-related deterioration of cell populations that express cholinergic receptors and acetylcholine-producing nuclei progresses, even cholinergic compensation is no longer sufficient to maintain "normal" cognitive performance, and noticeable symptoms emerge, i.e. slowing, memory lapses, attention deficits, and more.

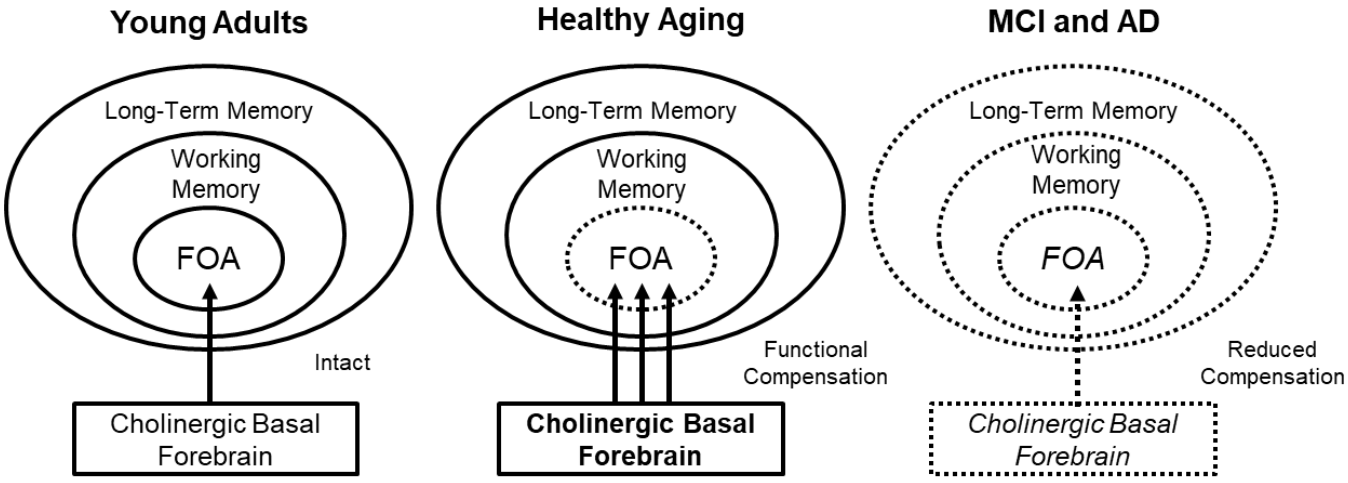


FIGURE 3: Model of the Basal Forebrain Cholinergic System Compensatory Functioning in Young, Older, and Impaired Older Adults. *FOA: Focus of attention*. Adapted from Dumas and Newhouse, (2011).

The PASA effect, or “posterior-anterior shift in aging” describes a differential pattern of BOLD activity in older, versus younger individuals, whereas older adults must recruit increased activity from additional areas in the frontal and parietal cortices to accomplish the same level of performance possible in younger individuals, whose neural circuitry is more intact and efficient (Davis, Dennis et al. 2008). This process may originate from increased activity output of the cholinergic receptor system, as it has been shown that individuals in the early stages of memory loss may show increased activity, connectivity and volume of the basal forebrain and medial temporal gray matter (Ikonomovic, Mufson et al. 2003, Butler, Harvey et al. 2018).

Leveraging the Cholinergic System as a Cognitive Treatment Target

With how closely the cholinergic system las been linked to cognitive ability, this receptor system has become one of the primary targets of medications intended to maintain cognitive performance, or at the very least, slow down the progression of inevitably worsening symptoms particularly in those who are experiencing pathological, rather than “normal” progression of age-related cognitive deficits. Acetylcholinesterase inhibitors, such as donepezil, rivastigmine, tacrine, galantamine, and others—function by blocking the acetylcholinesterase enzymes that rapidly metabolize endogenous acetylcholine, thus increasing the availability of the molecule to reach the receptors (Giacobini 2004, Kihara and Shimohama 2004). Though these types of medications only have shown modest success, they are still the first-line treatment for mild-moderate symptoms of Alzheimer’s disease (Potyk 2005). There have been additional agents attempting to increase cholinergic signaling that have had varying levels of success, although none have become clinically available as of yet.

The ease of administrating nicotinic agonists transdermally, orally, and nasally, has contributed to the considerable number of attempts to use nAChR agonists to attempt to exogenously improve cholinergic signaling and subsequently, cognitive performance. The degree of success of nicotinic

treatments has been linked to more advanced age and the baseline degree of impairment, (Picciotto and Zoli 2002), suggesting that nicotinic stimulation may be beneficial in those at higher risk for pathological aging processes like Alzheimer's, Parkinson's, and other dementing disorders. Nicotinic agonists have been shown to improve cognitive symptoms ranging from memory errors (Newhouse, Sunderland et al. 1988), attention and information processing (Jones, Sahakian et al. 1992), visual attention (Wilson, Langley et al. 1995) and verbal recall (Attaway, Compton et al. 1999) in adults with AD, and may have significant benefits in other age-related cognitive disorders that feature overlapping symptom profiles. The nicotinic system may indeed function as a target to improve certain cognitive processes, resulting in symptom improvement regardless of a certain diagnosis' specific underlying neurobiological processes.

Using muscarinic agonists to improve cognitive performance has been somewhat less successful than nicotinic treatment strategies, likely due to the difficulty in designing compounds that are selective for the receptor subtypes in the CNS (primarily M1/M4), and not for the other peripheral subtypes, that can contribute to side-effects (McArthur, Gray et al. 2010). There are a limited number of muscarinic agonist compounds being tested as treatments for AD symptoms, though more recent evidence suggests that activation of allosteric binding sites on the muscarinic receptors may be an alternate approach that would have reduced side effect liability (Kuduk and Beshore 2012).

Positive allosteric modulators (PAMs) enhance the binding of acetylcholine to receptors from a secondary binding site, keeping the molecules bound for longer, allowing them to increase post-synaptic activity. Development of PAMs has been a more recent approach, allowing for improved subtype specificity (Kuduk and Beshore 2012). PAMs selective for the M1/M4 receptors have not yet been widely assessed clinically, but by enhancing activation of neuronal mAChRs by exogenous or endogenous orthosteric ligands, PAMs may be an effective way to act indirectly on these receptors and benefit cognition.

To revisit the topic of this dissertation: research focused on the neuronal cholinergic system has identified numerous properties of this system that make it a useful research tool to better understand how neurobiological systems begin to deteriorate as a consequence of both age and pathological dysfunction. The flexibility of the cholinergic receptor system to finely tune its modulatory functions, its widespread influence on complex neural activity, and its reliable response to agonists and antagonists, make this a very useful candidate system to explore cognitive deficits across numerous disorders that impair cognition, that may involve cholinergic system damage even if that damage is not central to the etiology of the disorder. By using the cholinergic system as a tool to better understand the varying degrees to which certain domains of cognition are directly or indirectly a consequence of the disorder, we stand to gain a wealth of information not only about why certain domains are impaired, but also how we many also use this system for potential benefit, leading to treatment opportunities to maintain or improve cognitive abilities in these individuals in mid- to late-life.

CHAPTER V

COGNITIVE AGING IN THE HIV-POSITIVE POPULATION

Aged Phenotype in HIV: What Do We Know?

In the nearly 40 years since the emergence of the HIV virus, the field has made significant advances in the treatment of the disease, including awareness, prevention, detection, novel and more effective treatments, standard rigorous monitoring of patients, and more. Their successes have led to the current question facing HIV researchers and providers: what unique challenges does the aging process pose to an individual who has been living HIV-positive for five, ten, or in some cases, up to twenty years, and what further challenges lie ahead as they continue to live relatively healthy lives?

Older adults with HIV face many of the same symptoms of aging experienced by the HIV-negative population, however in most of the literature, there is a significant difference in the emergence, severity, and incidence of these symptoms, aging HIV-positive persons at a higher rate (Horvath and Levine 2015). Several studies have found excessive age-related changes to body composition, metabolism, and peripheral inflammation in the older HIV-positive population (Onen, Overton et al. 2010). HIV-positive status confers a significantly higher risk of cardiovascular dysfunction, such as hypertension (Singer, Valdes-Sueiras et al. 2013, Armah, Chang et al. 2014, Martin-Iguacel, Negredo et al. 2016, van Zoest, Wit et al. 2016), myocardial infarction (Friis-Moller, Sabin et al. 2003, Triant, Meigs et al. 2009, Freiberg, Chang et al. 2013) and elevated lipids and body fat (Riddler, Smit et al. 2003, Erlandson, Reynolds et al. 2014, Guaraldi, Lonardo et al. 2017). Two classes of ART medications, protease inhibitors and non-nucleoside reverse transcriptase inhibitors have been shown to increase cardiovascular disease risk in older HIV-positive patients, especially when combined in the same regimen (Friis-Moller, Weber et al. 2003). Increased peripheral markers of inflammation such as interleukin-6 (IL6) (Kuller, Tracy et al. 2008, French, King et al. 2009, Neuhaus, Jacobs et al. 2010) and

oxidative stress (Masia, Padilla et al. 2016), can also contribute to the accelerated physiological "weathering" experienced in even undetectable, asymptomatic older adults with HIV (French, King et al. 2009). Though these physical changes occur primarily in the periphery, increased stress on the cardiovascular system and elevated inflammatory markers due to HIV, ART, or both, can subsequently have deleterious effects on cognitive integrity.

Neurocognitive performance in the HIV-positive population is consistently lower than the HIV-negative population across several different domains, to varying degrees. As previously discussed, cognitive deficits in HIV may result from the combination of several factors: infection and subsequent death of astrocytes and microglia, indirect injury and apoptosis of neurons resulting from the death of glia, neurotoxic effects of ART, and persistent inflammatory microglial response to cellular and viral debris. As in the periphery, CNS markers of aging are also increased in HIV patients, and by some estimates an HIV-positive brain may be aging faster than HIV-negative brains (Levine, Quach et al. 2016, Cole, Underwood et al. 2017)

Neurocognitive and Behavioral Overlaps Between HAND and Cholinergic Dysfunction

From a structural perspective, the wide-spread projections of the basal forebrain cholinergic system, combined with the subcortical pattern of neurodegeneration caused by HIV, suggests that there may be regions of the brain where HIV-related damage overlaps with age-related, cholinergic axon and/or receptor loss that may exacerbate the resulting cognitive impairments. Many of the structures damaged by HIV are subcortical (Moore, Masliah et al. 2006), such as the thalamus, hippocampus, and are also known to highly express cholinergic receptors (Perry, Smith et al. 1989, Court, Lloyd et al. 1997, Graham, Martin-Ruiz et al. 2002, Gotti and Clementi 2004). Another primary target of HIV-related neurodegeneration is the deep frontal white matter tracts, some of which are those connecting the basal forebrain to cortical and subcortical targets (Chang, Wong et al. 2008, Kuper, Rabe et al. 2011, Watson, Busovaca et al. 2017)

Cognitive Domains	Cholinergic Dysfunction	HIV-Associated Neurocognitive Disorders
Attention/Concentration	+++	+++
Executive Functioning	++	+++
Psychomotor Speed	+++	++
Working/Verbal Memory	++	++
Verbal Fluency	+	+
Visuospatial Learning/Memory	++	+
Vigilance	+	-
Episodic Memory	++	++

TABLE 2: Commonly Affected Domains of Cognition Resulting from Cholinergic Dysfunction and HIV-Associated Neurocognitive Disorders (HAND). "Cholinergic Dysfunction" also represents the known effects of anticholinergic medications.

Behaviorally, there is overlap in the cognitive deficits that present with cholinergic blockade (which resembles cognitive aging) and those that present in HAND (Table 2). In order to assess whether these deficits may be a direct result of damage to the cholinergic system, the use of cholinergic antagonists can help to evaluate the relative contribution of cholinergic impairment to the symptoms of HAND; whether the cholinergic system is a primary vulnerable system, or if damage to the cholinergic system only accounts for a portion of the symptoms. Differential response to cholinergic blockade is a useful methodological probe to assess cholinergic "tone", or the integrity of the cholinergic receptor system. Increased response to one drug or the other or the combination of both relative to placebo or relative to the HIV-negative may indicate alteration of muscarinic and nicotinic receptor system function due to HIV serostatus, age, or a combination of the two.

Few studies so far have explored the cholinergic system specifically in regards to HIV-cognitive functioning, but there have been some related studies that may provide some insight. It has been estimated that nearly 60% of HIV-positive adults are current or previous smokers (Reynolds 2009,

Mdodo, Frazier et al. 2015) nearly double the prevalence of nicotine use in the HIV-negative population (West 2017). Nicotine, a potent receptor agonist of the so-called nicotinic acetylcholine receptor subtypes (nAChRs), has previously been shown to be a pro-cognitive agent, particularly in individuals experiencing early, low-level cognitive dysfunction (Le Houezec, Halliday et al. 1994, Levin, Conners et al. 1996, White and Levin 1999, Howe and Price 2001, Hahn, Ross et al. 2007, Newhouse, Kellar et al. 2012, Gandelman, Kang et al. 2018). In a study of smoking habits and cognitive performance amongst HIV-positive women, it was found that those with a history of nicotine usage had improved cognitive performance, relative to non-smokers (Wojna, Robles et al. 2007). The authors hypothesize that the heavy usage of nicotine in the HIV-positive population may be an unwitting form of cognitive self-medication, possibly counteracting some of the subtle cognitive changes of HAND by engaging the compensatory functionality of the system, as it has been shown that the presence of nicotine and endogenous acetylcholine results in higher concentrations of nAChRs being activated, improving signal transduction at cholinergic synapses (Marks, Stitzel et al. 1987, Pomerleau 1992, Wylie, Rojas et al. 2012, Niemegeers, Dumont et al. 2014).

There is also some neurobiological evidence that the cholinergic system may be particularly vulnerable to HIV-related cell damage, resulting from the presence of the viral surface protein gp120 (Bracci, Lozzi et al. 1992, Banks, Robinson et al. 2005, Agrawal, Louboutin et al. 2010). Once an infected cell has begun to produce HIV proteins, enzymes, and other materials, the “extra” particles that are not assembled into a viral capsule can still cause damage to surrounding cells due to other interactions. The Tat protein, which functions inside the cell to upregulate the host-cell’s translation of viral DNA into viral proteins, has been shown to be neurotoxic to both neurons and glia (Marciniak, Calnan et al. 1990, Zauli, Secchiero et al. 2000, Banks, Robinson et al. 2005), enhancing the persistent neuroinflammation that leads to cognitive dysfunction and HAND. Additionally, spectroscopy studies have shown that due to a region on the gp120/gp41 protein complex that is similar to the acetylcholine molecule’s binding region (Freed, Myers et al. 1990), the gp120 protein has affinity not just for the CD4

receptor, but also for the homomeric α7-nAChR (Bracci, Lozzi et al. 1992, Ballester, Capo-Velez et al. 2012). The high binding potential to these ion channels can disrupt the carefully controlled modulatory functioning of these receptors, allowing for an excessive Ca^{2+} influx, triggering apoptotic cell death (Ciardo and Meldolesi 1993, Kaul and Lipton 1999). Gp120 may also facilitate the reproduction of reactive oxygen species that increase apoptotic loss of dopamine neurons in the substantia nigra, causing some of the Parkinsonian-like motor impairments that are present in HAND (Nosheny, Bachis et al. 2006, Agrawal, Louboutin et al. 2010). The deleterious neurobiological effects of gp120 protein, as well as the cognitive dysfunction that results from the loss of these cholinergic inputs, can be mitigated with administration of a nicotinic agonists (Gonzalez-Lira, Rueda-Orozco et al. 2006), which may be 1. simply out-competing the viral protein, 2. acting as a neuroprotective agent, or a combination of both.

CHAPTER VI

Developing and Examining a Model of Acquired Pathological Cognitive Aging

Background Summary

In prior chapters, I have reviewed a selection of literature discussing the neurobiology of cognitive aging, and how chronic HIV infection alters that phenotype. The introduction of the HIV virus into the body and subsequently the brain has long-term consequences for cognitive health, changing not only the trajectory of cognitive decline, but also the severity, as glial and neural cells are lost due to a cycle of damaging, persistent inflammation. While it is possible to see gross changes in brain tissue morphology, connectivity, and metabolism resulting from HIV using MRI and other imaging modalities, these methods do not necessarily allow researchers to fully understand how critical neurotransmitter and receptor systems are disrupted by the combination of age-related and HIV-related pathology.

The cortical acetylcholine receptor is a widespread neurotransmitter system, projecting from four small nuclei to virtually the entire brain, modulating cognitive functioning by increasing or decreasing release of acetylcholine and other neurotransmitters to focus attention, integrate signals from multiple smaller networks, and optimize higher level cognitive performance. The functioning of this neurotransmitter system may change over time, contributing to the symptoms commonly associated with aging: memory lapses, attentional errors, psychomotor slowing, and more.

As the HIV epidemic is roughly four decades old, it is only in the last 10 years or so that many survivors of the initial outbreak are entering late-life, and with prevention and treatment strategies improving, the average age of the HIV-positive community will likely continue to rise. The older these individuals get, understanding what aging looks like in this population versus the HIV-negative population becomes a more pressing issue, with many unanswered questions to be explored. In

particular, there are indications that the progression of cognitive aging occurs faster in adults living with HIV, although it is not yet understood whether these processes are additive or interactive. To this end, the focus of this dissertation research was to examine the relative functioning of the neuronal acetylcholine receptor system in middle-aged and older HIV-positive adults, to understand the neurochemical underpinnings of the cognitive aging process in adults living with HIV.

Overall Rationale and Research Aims

Prior research on the cholinergic receptor system has shown that short-acting anticholinergic medications can temporarily reproduce the cognitive phenotype of aging, and the magnitude of their effect is dependent on the overall integrity of the system. Older adults and adults with other disorders causing neurological impairments reliably are more sensitive to these medications, as they may have fewer cholinergic projections and functioning receptors relative to younger, neurocognitively intact individuals. In this way, cholinergic antagonists, such as mecamylamine and scopolamine, can be used as a temporary cholinergic “lesion”, to better understand how the loss of these receptor systems can affect attention and memory performance as individuals age.

Although damage to the cholinergic system may not be the primary pathological outcome of HIV-infection, understanding how the cholinergic system is affected by HIV and aging together will provide valuable insight into the symptoms of HAND, and inform prognostic decisions as these adults continue to age into late-life. The goal of this research was to ascertain if there is a deficit in cholinergic functioning in the brains of adults living with HIV, contributing to the presence and severity of HIV-Associated Neurocognitive Disorder symptoms and age-related cognitive impairments earlier than is seen in HIV-negative persons. The following chapters will discuss two completed research studies intended to address these questions.

Study I: Objective and Subjective Memory Impairments in HIV-Positive and At-Risk Adults

This pilot survey study was designed to determine feasibility and sensitivity of a brief cognitive battery to identify and monitor subjective and objective cognitive abilities in a sample of HIV-positive clinic patients, and HIV-negative controls. Participants were approached and screened during the course of their normal visit to the Vanderbilt Comprehensive Care Clinic and Vanderbilt Medical Center's outpatient Clinics (Nashville, TN), and consented to completing a short battery of demographic and cognitive measures.

Specific Aim 1: To evaluate the relationship between objective and subjective measures of cognitive ability. In a population with increased risk of moderate to severe cognitive impairments at an earlier stage than HIV-negative patients, we sought to evaluate whether cognitive complaints (subjective memory complaints) had a meaningful correlation with objective measures of cognitive ability in HIV-positive patients, relative to their HIV-negative counterparts.

Specific Aim 2: To determine feasibility of administering a short, routine cognitive battery in a clinic setting. Though prior literature has shown the prevalence of HIV-Associated Neurocognitive Disorders to affect nearly 60% of HIV-positive adults in their lifetime, routine cognitive monitoring is not commonly performed in a clinic setting until the patient's symptoms begin to affect daily life. We designed a short battery to be completed within the setting and time-constraints of a regular clinic visit, to evaluate whether this could be a worthwhile addition to routine clinical HIV patient monitoring.

Study II: Cholinergic Correlates of Impaired Cognitive Ability in HIV-Associated Neurocognitive Disorders (HAND)

This double-blind, placebo-controlled, cognitive challenge study was designed to evaluate the relative contribution of the cholinergic system to age-related symptoms of cognitive impairments in

adults living with HIV, in comparison to an age-matched cohort of healthy HIV-negative participants. Participants were recruited from the Vanderbilt Comprehensive Care Clinic, Vanderbilt Medical Center outpatient Clinics, and HIV/AIDS outreach organization Nashville CARES, and screened and consented to complete four research study days involving a comprehensive cognitive battery after the administration four different possible “Challenge” medications: High Dose (20 mg) Mecamylamine, High Dose (5 mcg/kg) Scopolamine, a Low-Dose (10 mg/2.5 mcg/kg) combination of Mecamylamine and Scopolamine, or a double placebo.

Specific Aim 1: To evaluate the relative effects of acute nicotinic receptor blockade, muscarinic blockade, or a combination, on cognition in HIV- positive adults and HIV-negative adults. I hypothesized that an interaction between HIV-status and the effects of a cholinergic antagonist would produce a more severe impairment in the HIV-positive cohort relative to the HIV-negative cohort, indicating a baseline cholinergic deficit resulting from the on-going effects of chronic CNS HIV-infection.

Specific Aim 2: To evaluate the effect of age on the relative difference of cholinergic antagonist effects on cognition in HIV-positive adults compared to HIV-negative adults. As it is known that in the HIV-negative population, older adults are more sensitive to the effects of anti-cholinergic medications, I hypothesized that in the HIV-positive group, the effect of older age would result in more significant impairments in response to the study medications, relative to the younger HIV participants and the HIV-negative participants.

Potential Impacts

Better understanding of how cellular-level changes in the brain produce phenotype of HIV-HAND have several possible impacts for this community. Most obviously, confirming the involvement of the cholinergic system in the deficits of HAND provides an opportunity to evaluate the usefulness of

cholinergic agonists for the improvement of cognitive symptoms. Several novel muscarinic and nicotinic agents have been discovered and become the focus of clinical trials in various disorders; Mild Cognitive Impairment, Alzheimer's Disease, Geriatric Depression, and more, with promising results. As there is significant overlap between these disorders of pathological aging, and HAND, it is reasonable that these kinds of agents may also provide cognitive and mood benefits for a population facing a considerable risk of both. There are currently no approved treatments for the symptoms of HAND, and though a few studies have shown modest improvements with the administration of acetylcholinesterase inhibitor medications, further studies that consider the full breadth of cholinergic treatment possibilities in disorders with similar symptom profiles should continue.

The intersection of age- and disease-related changes to neurocognition is a complex one, but understanding how the cholinergic system is involved in these processes may answer many important questions for both clinicians and patients living with these symptoms. The considerable overlap in cognitive symptoms between these models suggests that there may be a cholinergic component to the cognitive impairments across them. Though cholinergic treatments may not address HIV in the brain directly, they still may have merit to improve cognitive symptoms, and subsequently quality of life; especially as targeted disease treatments improve longevity such as in HIV. The cholinergic system may function as a global compensatory system, improving cognitive functioning by modulating other related neurotransmitter systems in an attempt to maintain performance. The cholinergic neurotransmitter system may respond positively to cholinergic treatments not only because there is a disease-specific injury to the neurotransmitter system, but instead, because it may function as a "universal target" to improve attention and cognitive performance.

The following chapters will detail two completed research studies aiming to answer some of these questions, evaluating the involvement of the cholinergic receptor system in the cognitive deficits of HIV-Associated Neurocognitive Disorders. Considering cholinergic system as a universal target,

understanding its role in cognitive impairments arising from chronic HIV infection will increase understanding of potential mechanisms to maintain or enhance cognitive abilities by leveraging this system as adults living with HIV continue to survive into old age.

CHAPTER VII

STUDY I: OBJECTIVE AND SUBJECTIVE MEMORY IMPAIRMENTS IN HIV-POSITIVE AND AT-RISK ADULTS

Rationale

As discussed in Chapter 3, cognitive aging of the brain is a process that may be accelerated by the additional burden of HIV-related damage to the brain, which in turn may cause more noticeable, earlier symptoms in HIV-positive adults relative to their HIV-negative counterparts. The goal of this first small study was twofold: To understand whether a relatively short cognitive battery could feasibly be administered in a normal clinical setting, and to survey the spectrum of cognitive deficits in the Vanderbilt Medical Center Comprehensive Care Clinic population and how well they correlate with self-reported symptoms, or so-called Subjective Memory Complaints (SMCs).

Subjective memory, or a patient's own appraisal of their memory functioning, is easily collected but surprisingly little used. In the cognitive aging literature, the concept of using the patient's own opinion of their memory status is controversial, with many studies finding little to no association with actual cognitive performance in cognitively "normal" HIV-negative middle aged and older adults (Howieson, Mattek et al. 2015, Pennington, Hayre et al. 2015, Thompson, Henry et al. 2015). Studies have found however that in adults in the early stages of cognitive impairment, subjective memory does have some predictive value. Subjective memory was shown to correspond with informants' ratings of memory ability in adults with Mild Cognitive Impairment (MCI) or younger dementia patients (Abner, Kryscio et al. 2015, Buckley, Saling et al. 2015, Salem, Vogel et al. 2015), and was also shown to correlate with their self-reported ratings of mood symptoms (Yates, Clare et al. 2015). Furthermore, subjective memory ratings may strongly predict those who will develop more severe cognitive impairments, particularly in adults over the age of 60 (Ronnlund, Sundstrom et al. 2015). Subjective memory impairment has also been specifically associated with impairment in verbal episodic memory

performance (Gifford, Liu et al. 2015), which may indicate that verbal episodic memory tasks may be sensitive to measurement in the early stages of memory impairment.

Subjective memory complaints have been evaluated as a predictor of cognitive ability in at least two studies of healthy HIV-positive adults, Though they did not find an association between cognitive performance and subjective memory, instead finding strong correlations between subjective memory and depressive symptoms (van Gorp, Satz et al. 1991, Au, Cheng et al. 2008), a result which has also been found in several studies of older HIV-negative adults (Small, Chen et al. 2001, Crane, Bogner et al. 2007, Bartley, Bokde et al. 2012, Hulur, Hertzog et al. 2014). It is unclear whether subjective memory impairments predict objective change in cognitive ability, or if, as one study found, measurable cognitive impairments predict changes in self-reported appraisal of memory ability (Bassel, Rourke et al. 2002, Snitz, Small et al. 2015).

The sensitivity and thoroughness of the subjective memory measures in these studies vary, in some cases relying on the rating of a single question (such as “how would you rate your memory?”) suggesting that clearer results may emerge with the use of a different, more comprehensive instrument. The Memory Functioning Questionnaire (MFQ) with its more in-depth items may serve this purpose, as it has been previously validated to correlate with actual memory performance (Zelinski, Gilewski et al. 1990), and a measure with more items may be more sensitive to small changes in cognitive performance.

We hypothesized that subjective memory complaints would correlate with subtle objective memory impairments more significantly in HIV-positive adults than HIV-negative controls. If validated, this may help clinicians to identify those patients who may need to be monitored more closely for development of more severe cognitive symptoms, or who may be at higher risk for developing HAND.

Methods and Study Design

Study Participants

For this study, 35 HIV-positive adults and 21 HIV-negative adults were enrolled (total n=56). HIV-positive adults were approached for participation while waiting for their routine medical visits to the Vanderbilt Comprehensive Care Clinic (Nashville, TN) as well as via study advertisements posted in the offices of Nashville CARES, an HIV outreach organization in middle Tennessee. HIV-negative control participants were recruited via flyers and advertisements dispersed throughout the Vanderbilt University Medical Clinics and campuses, to provide a comparable clinical sample to the HIV-positive participants. HIV-positive and -negative groups were similar with respect to race, sex, age, and education level (Table 3).

Participants were asked about their current and past substance use, and any participants endorsing current active substance abuse disorders (alcohol, cocaine, opiates) were excluded from participation. HIV-positive participants who were not currently on a stable ART regimen were excluded. Written informed consent was obtained from all participants included in the study, as approved by the Vanderbilt University Institutional Review Board and Human Research Protection Program. After consent, participants were asked to provide demographic information and medical history. After completion of all tasks, subjects were each compensated for their time and participation.

Memory and Mood Measures

The Memory Functioning Questionnaire (MFQ) is a 64-item self-report survey of current memory problems, each rated on a scale from 1-7. With symptom categories such as “frequency of forgetting”, “seriousness of forgetting”, and “mnemonic usage”, the measure is intended to assess subjective memory in middle aged and older adults (Gilewski and Zelinski 1988). The Beck Depression Inventory (BDI) is a 21-item self-report questionnaire to measure incidence and severity of depressive symptoms that the subject may have recently experienced, rated on a scale from 0-3 (Beck, Ward et al.

1961). The Beck Anxiety Inventory (BAI) is a 21-item self-report questionnaire to measure incidence and severity of anxiety symptoms that the subject may have recently experienced, rated on a scale from 0-3 (Beck, Epstein et al. 1988). For the MFQ, BDI and BAI, participants were asked to rate each item for the previous month (30 days), including their study day.

	HIV-Negative	HIV-Positive	p-value
	N=21	N=35	
Gender (% Male)	47.6	57.1	0.584
Race (% White)	61.9	42.9	0.270
Years of Education	15.61	14.0	0.083
Age	46.6 (13.5)	49.1 (11.5)	0.459
Minimum-Maximum Age	23-69	26-76	--
CD4 Nadir	--	395.6 (209.6)	--
CD4 Current	--	754.3 (494.8)	--
MFQ Score	286.4 (54.9)	275.1 (87.6)	0.600
BDI Score	13.7 (8.1)	18.6 (11.0)	0.081
BAI Score	12.9 (14.4)	18.2 (13.3)	0.165
Detection Accuracy	1.48 (0.11)	1.38 (0.25)	0.087
Identification Accuracy	1.37 (0.14)	1.30 (0.25)	0.344
One-Card Learning Accuracy	0.98 (0.09)	0.88 (0.07)	**< 0.001
Groton Maze Duration (s)	56.92 (22.52)	114.56 (64.16)	**< 0.001
Groton Maze (moves per second)	0.56 (0.20)	0.34 (0.18)	**< 0.001
SRT-Total Recall	75.00 (28.06)	49.18 (16.44)	**< 0.001
SRT-Total Recall (Delay)	9.52 (5.06)	5.61 (3.37)	*0.002
SRT-Total Consistency	7.38 (0.81)	7.00 (0.79)	0.098
SRT-First 4 Recall	31.52 (12.08)	20.96 (6.03)	**< 0.001
SRT-Last 4 Recall	43.47 (15.50)	28.21 (11.09)	**< 0.001
SRT-First 4 Consistency	7.35 (3.69)	10.47 (1.73)	**< 0.001
SRT-Last 4 Consistency	7.40 (3.23)	4.40 (1.93)	**< 0.001

TABLE 3: Demographic Characteristics and Cognitive Battery Scores, by Serostatus Group

Cognitive Battery

The Selective Reminding Task (SRT) is an episodic verbal memory task (Buschke 1973), where participants are read a list of sixteen (16) semantically unrelated, low and high imagery words, and asked to recall as many as possible (no time limit). Participants completed eight (8) recall trials, and between each trial the experimenter reads only the words that the participant failed to recall, and does not reinforce the words successfully recalled. Recall, and Recall Consistency (defined as number of words successfully recalled in two (2) sequential trials without reinforcement) are recorded. Participants also completed a Delayed Recall trial, after the CogState Battery (approximately 25 minutes). The CogState Cognitive Assessment System is a computerized battery designed to quickly and comprehensively assess cognitive status (Fredrickson, Maruff et al. 2010). We used four tasks previously and successfully used in an HIV-positive population (Cysique, Maruff et al. 2006, Boivin, Busman et al. 2010, Thiyagarajan, Garvey et al. 2010, Winston, Puls et al. 2012) to assess neuropsychological function. Tasks included The Groton Maze (executive functioning and visuospatial problem solving), Detection (psychomotor functioning and reaction time), Identification (visual attention), and One-Card Learning (visual learning, working memory). These cognitive domains have been routinely evaluated in prior studies of cognitive ability in HIV-positive adults (Heaton, Franklin et al. 2011, Blackstone, Moore et al. 2012).

Statistical Data Analysis

Group means were calculated for age, BDI and BAI Scores, and MFQ Score. Group means were compared between HIV-positive and HIV-negative groups for Cognitive and Mood measures using independent samples t-tests. In the HIV-positive group, mean CD4 T-cell nadir and current CD4 T-cell counts were obtained from patient medical records based on evidence from the CNS HIV Anti-Retroviral Therapy Effects Research “CHARTER” study indicating that CD4 nadir count is a significant predictor of risk for cognitive impairments in HIV positive adults (Ellis, Badiee et al. 2011). “Nadir” CD4 count was defined as the first CD4 count recorded in the participant’s medical record after a positive

HIV-test was confirmed, and “current” CD4 count was defined as the most recently obtained CD4 count in the patient’s medical record, within the past 30 days. For the Selective Reminding Task, Total Recall and mean trial-to-trial Consistency for the full eight-trial task (SRT-T) were calculated, as well as Total Recall and mean trial-to trial Consistency for first four (SRT-F) and last four (SRT-L) trials. Approximately 5 minutes after completing the last trial of the SRT, participants completed a delayed recall trial, with no reinforcement of the word list. These episodic memory measures were calculated for each participant, and averaged within serostatus group.

SRT measures were compared between serostatus groups using independent samples t-tests. “Accuracy” on the CogState tasks was defined as an arcsine transformation of the proportion of correct to incorrect responses on each task, and group differences in performance was calculated using independent samples t-tests. Pearson Correlations (2-tailed) were calculated to evaluate associations between MFQ score and the following factors: age, BDI/BAI score, SRT-T, SRT-F and SRT-L performance measures, CogState performance accuracy measures; and in the HIV-positive group, additionally CD4 nadir count. Multiple regression analysis was conducted to evaluate whether MFQ and BDI scores would predict total recall performance on the episodic memory task.

Results

Group means and standard deviations for the descriptive, behavioral, and cognitive data for both study groups can be found in Table 3. There was no significant difference in mean age between serostatus groups ($t= -0.745$, $p= 0.459$). Neither mean BDI score nor mean BAI score were significantly different between groups (BDI: $t= -1.78$, $p= 0.081$; BAI: $t= -1.407$, $p= 0.165$). Mean MFQ score was also not significantly different between groups ($t= 0.528$, $p= 0.600$).

HIV-negative participants had significantly better episodic memory performance (higher total words recalled) across the total task (SRT-T) compared to HIV-positive participants ($t=4.270$, $p< 0.001$),

while total (8-trial) recall consistency was not significantly different between groups (t= 1.685, p = 0.098), as shown in Figure 4. HIV-negative participants also recalled significantly more words on the delayed recall trial compared to HIV-positive participants (t= 3.330, p= 0.002). When task performance was split between the first four (SRT-F) and last four (SRT-L) however, recall and recall consistency were significantly different between serostatus groups on both the first four (SRT-F t=4.026, p< 0.001; SRT-F Consistency t= -4.21, p< 0.001) and last four (SRT-L t=4.218, p< 0.001; SRT-L Consistency t=4.275, p< 0.001) trials of the task, shown in Figure 5. Additionally, paired t-tests revealed that while differences in recall consistency were not statistically significant between SRT-F and SRT-L in the HIV-negative group (t= -0.38, p= 0.970), recall consistency was significantly different between SRT-F and SRT-L in the HIV-positive group (t= 10.340, p< 0.001).

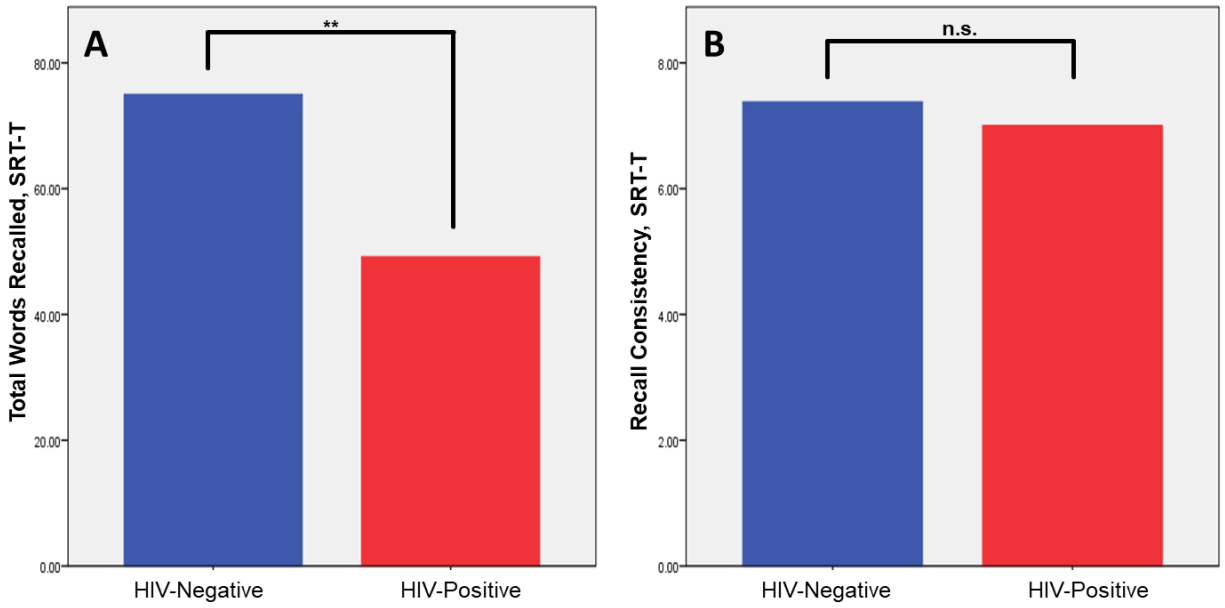


FIGURE 4: Selective Reminding Task – Total Recall and Consistency Performance, by Serostatus

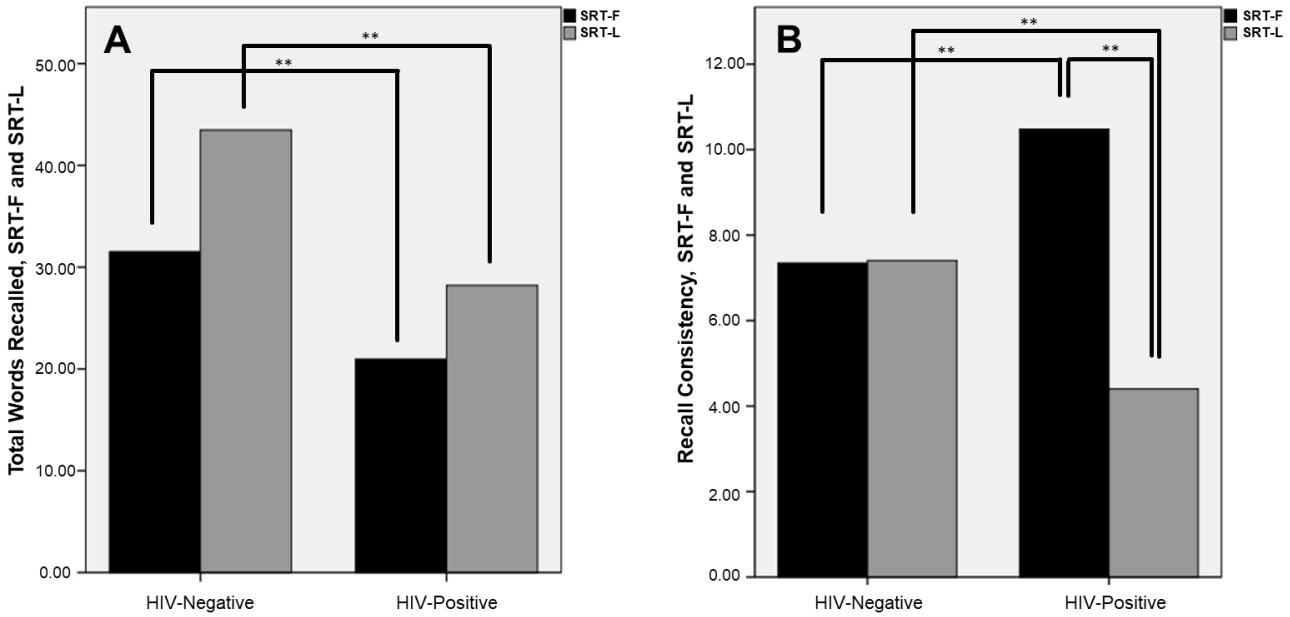


FIGURE 5: Selective Reminding Task -- First 4 Trial/Last 4 Trial Recall and Consistency Performance, by Serostatus

HIV-negative participants performed significantly better on the episodic recognition task from the CogState, the One Card Learning Task (higher accuracy) compared to the HIV-negative participants ($t= 4.07$, $p< 0.001$). On the Groton Maze, the HIV-negative group completed the maze significantly faster than the HIV-positive group ($t= -3.848$, $p< 0.001$), and completed significantly more moves per second ($t= 4.182$, $p< 0.001$). No other performance measures of the CogState battery were significantly different between serostatus groups.

MFQ score was significantly negatively correlated with both BDI and BAI ratings in both the HIV-negative and HIV-positive group. As self-reported depression and anxiety symptoms increase, subjective memory rating score decreases, consistent with previous literature in both the HIV-negative (MFQ x BDI corr= -0.618, p=0.003; MFQ x BAI corr= -0.466, p<0.001) and HIV-positive (MFQ x BDI corr =-0.579, p=0.03; MFQ x BAI corr = -0.630, p<0.001) groups.

MFQ score was significantly positively correlated with SRT-T total words recalled in both the HIV-negative (MFQ x SRT-T corr= 0.499, p= 0.021) and HIV-positive (MFQ x SRT-T corr= 0.432, p= 0.012) groups, though delayed recall trial performance was only significantly correlated with MFQ score in the HIV-positive group (MFQ x SRT-T Delay corr= 0.548, p= 0.002), but not the HIV-negative group (MFQ x SRT-T Delay corr= 0.337, p= 0.136) shown in Figure 5.

There were no significant correlations between mood ratings and age in either the HIV-negative group (BDI x age corr= 0.386, p= 0.084; BAI x age corr = 0.151, p= 0.514) or HIV-positive group (BDI x age corr= -0.026, p= 0.880, BAI x age corr= -0.184, p= 0.291). Age was also not significantly correlated with MFQ score in either the HIV-negative (age x MFQ corr= -0.294, p= 0.195) or the HIV-positive group (age x MFQ corr= 0.068, p= 0.697). Age was significantly negatively correlated with SRT-T recall and delayed recall in the HIV-negative (age x SRT-T corr= -0.492, p=0.024; age x SRT-T Delay corr= -0.546, p= 0.010), but not the HIV-positive group (age x SRT-T corr= -0.151, p=0.402; age x SRT-T Delay corr= -0.115, p= 0.546). There were also no significant sex differences in MFQ scores in either serostatus group, and no significant correlations between current or nadir CD4+ cell count and any cognitive measures.

MFQ score was significantly positively correlated with episodic recognition performance, as measured by the One-Card Learning (OCL) task of the CogState battery, in the HIV-positive group (MFQ x OCL corr = 0.414, p = 0.025) but not in the HIV-negative group (MFQ x OCL corr= 0.105, p = 0.658), shown in figure 5. Speed on the Groton Maze task was also significantly different between groups, with HIV-negative participants completing the task significantly faster than the HIV-negative group (t= -3.85, p< 0.001) and with significantly more moves per second (t= 4.182, p<0.001). Groton Maze performance was not correlated with MFQ score in either group, and no other measures of the CogState were significantly different between serostatus groups, or significantly correlated with subjective memory scores.

Despite statistically insignificant differences in BDI score between serostatus groups, regression analysis showed that with a model including MFQ score and BDI score, BDI was the most significant predictor of SRT-T recall performance in the HIV-negative group ($F(2,18)=6.36$, Sig = 0.008, BDI beta = -0.516, MFQ beta = 0.180) whereas in the HIV-positive group, MFQ score was the most significant predictor of SRT-T recall performance ($F(2,30)=3.45$, Sig = 0.045, BDI beta = 0.23, MFQ beta = 0.446).

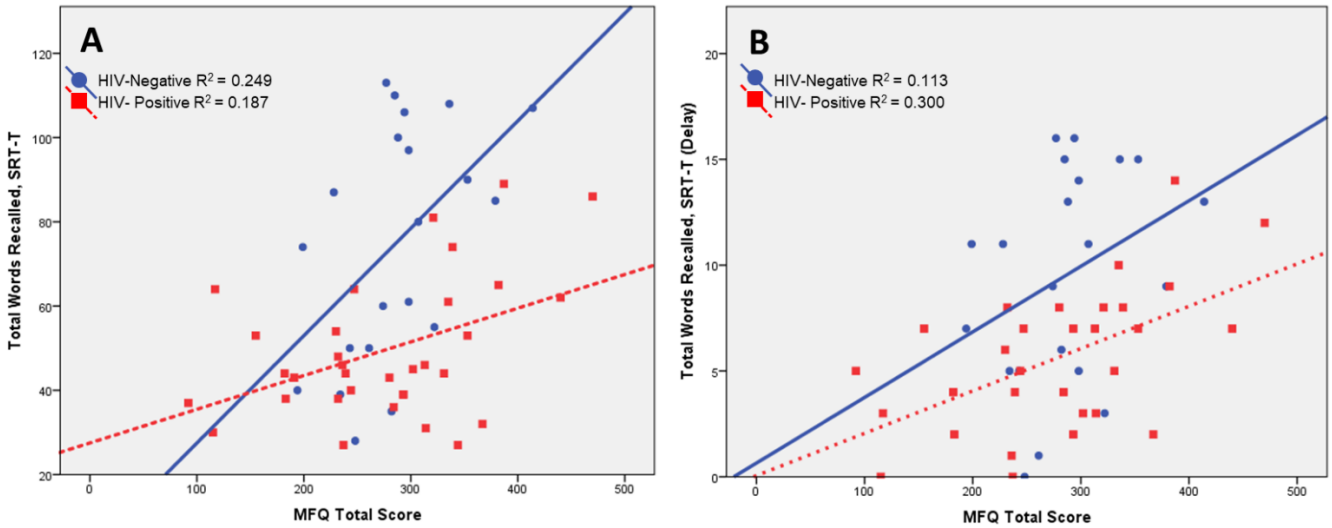


FIGURE 6A, B: Correlation between Memory Functioning Questionnaire Score and Selective Reminding Task -- Immediate Recall and Delayed Recall performance

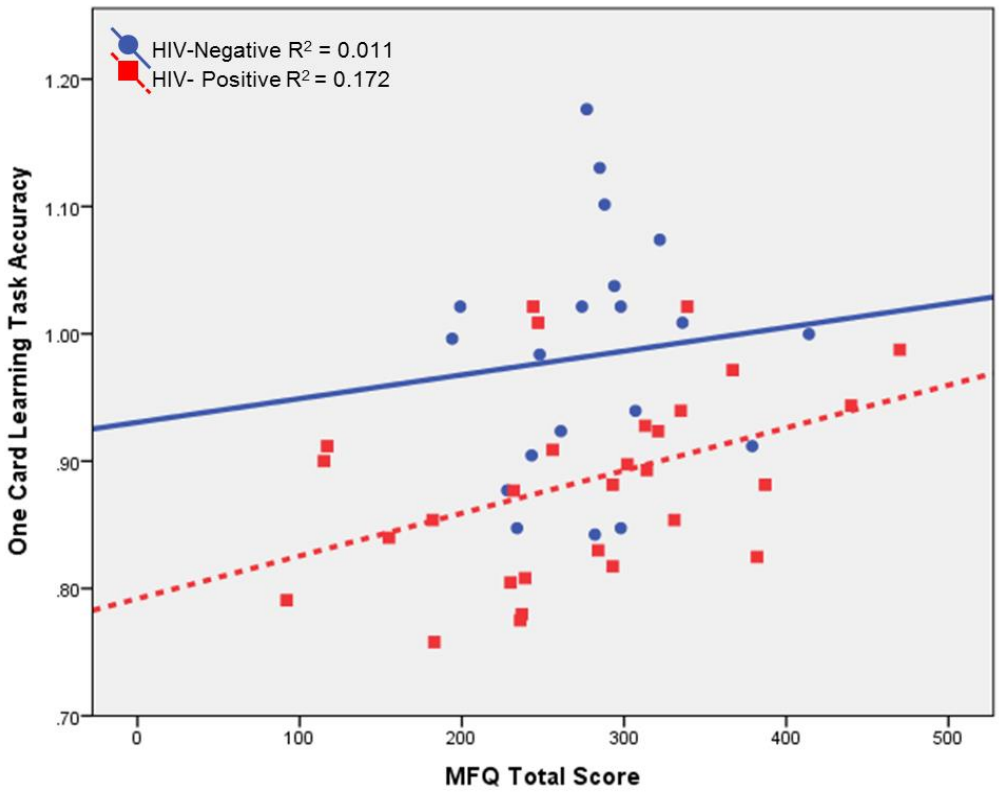


FIGURE 7: Correlation between Memory Functioning Questionnaire Score and CogState One-Card Learning Task Performance

Discussion

Consistent with HIV cognition literature, the HIV-positive participants in this study performed significantly worse at both verbal episodic memory and recognition tasks compared to HIV-negative matched controls, with lower word recall and lower recall consistency on the SRT Task and poorer stimulus recognition on the OCL task. On the SRT task, the differences in recall consistency between the early and late trials of the task may reflect impairment not only in memory recall, but also in transferring encoded information successfully from short term to long term storage. Previous studies have linked episodic memory failures in HIV to the deterioration of fronto-striatal white matter and loss of hippocampal volume (Castelo, Sherman et al. 2006, Pfefferbaum, Rogosa et al. 2014) impairing working memory and memory storage and retrieval, which may be the underlying mechanisms in this HIV-positive group.

BDI, BAI, and MFQ scores were not significantly different between the HIV-positive and HIV-negative groups, and MFQ score was significantly correlated with BDI and BAI in both groups. Similar results have been shown in prior studies (van Gorp, Satz et al. 1991, Au, Cheng et al. 2008), with differences in subjective memory ratings not corresponding with memory performance. However, in this study, while subjective memory score was significantly correlated with objective episodic memory performance in the HIV-positive group, depression was not the best predictor of performance variance in SRT performance in the HIV-positive group. For these adults, subjective memory score on the MFQ accounts for the most significant portion of SRT performance variance. This may indicate that in HIV-positive adults, subjective memory complaints may be a more salient marker of actual early cognitive deficits compared to depression symptoms. Thus, rating of memory complaints may deserve monitoring by medical professionals, as these individuals are at increased risk for developing more extreme cognitive impairments with age.

Previous studies have found that depressive and anxiety symptoms are associated with both subjective memory and objective performance (Lineweaver and Brolsma 2014, Rowell, Green et al. 2015), however a unique property of this study sample was the similar depression ratings in the two groups. Because of the known higher proportion of mood disorders in the HIV-positive population, it may be more clinically relevant to compare HIV-positive populations with a control group that has worse mood ratings, to more clearly examine the effects of serostatus alone. However, with these relatively high depressive symptoms in both groups, this data may not be predictive of results in HIV-positive individuals with low symptoms of depression.

The results of this study correspond closely with emerging literature from aging and dementia literature, which point to subjective complaints of memory as a useful, non-invasive marker to identify the early stages of memory and cognitive impairment in adults experiencing pathological aging processes, versus those experiencing expected “normal” age-related changes to their cognitive abilities. This is an important distinction, since some studies of cognitively normal older adults have found little to no correlation between subjective memory complaints and objective performance (Fritsch, McClendon et al. 2014, Hulur, Hertzog et al. 2014, Abner, Kryscio et al. 2015, Dalla Barba, La Corte et al. 2015, Howieson, Mattek et al. 2015), whereas correlations have been found in adults with Mild Cognitive Impairment or Dementia diagnoses (Gifford, Liu et al. 2015, Thompson, Henry et al. 2015, Yates, Clare et al. 2015). The positive results in this study may also be due to the more extensive subjective memory rating measure used in this study, the MFQ. In prior studies subjective memory was assessed with a measure of less than 10 items, often a single item, whereas the MFQ’s more detailed query of various aspects of memory may give a more accurate portrayal of subjective memory impairment.

As expected in the HIV-negative group, higher participant age was significantly associated with fewer words recalled in the immediate and delay trials of the SRT task. Interestingly enough, we found

that age was only significantly correlated with SRT recall consistency in the HIV-positive group, and not with any other SRT measure. Our control group data is consistent with previous literature indicating that older age is significantly negatively correlated with cognitive performance in older HIV-negative adults, but in the HIV-positive individuals in this study, age was less important in predicting performance.

The design of this study was not sufficient to explore the many underlying biochemical factors of HIV infection and treatment that may affect cognitive performance and mood, nor was it appropriate to explore the interaction between aging and HIV. The relative penetrance of different ART regimens (Skinner, Adewale et al. 2009, Decloedt, Rosenkranz et al. 2015), the neurotoxic effects of some ART medications (Akay, Cooper et al. 2014, Underwood, Robertson et al. 2015), high prevalence of mood disorders (Silveira, Guttier et al. 2012, Dal-Bo, Manoel et al. 2015), history of substance abuse (Byrd, Fellows et al. 2011) as well as length and lifetime severity of HIV disease (Heaton, Franklin et al. 2015) are all factors that can negatively affect cognitive performance in aging HIV-positive adults, and these can fluctuate over time (Dawes, Suarez et al. 2008). However future investigation into the relative differences in subjective memory ratings when accounting for these differences in disease and treatment may be necessary to tailor cognitive monitoring to individual patients, i.e. using these different factors to further specify those with greater or fewer risk factors for early cognitive changes.

Clinical Implications and Management for Older Adults with HIV

With the knowledge that a substantial proportion of the HIV-positive population will develop some degree of cognitive impairment over the course of their life, it is important that cognitive evaluations become more widespread within HIV clinics. There currently isn't a consensus as to which measures are appropriate and sensitive to accurately detect individual cognitive changes over time. Many HIV clinics routinely provide biological, physiological, sociological and psychiatric monitoring of their HIV patient's well-being, in many cases combining a number of these various follow-ups into a single, comprehensive visit. Cognitive monitoring however, is a relatively new concept, and as this field

continues expanding, clinicians and researchers alike should consider how best to address on-going cognitive assessment, particularly in patients over the age of 40. Like many other facets of HIV-related health care, such as CD4 count, detectable vs. undetectable viral load, educating the patient on their risk for cognitive changes, what to expect, and when to alert their caregivers to changes, may be helpful to identify when further assessment and monitoring is warranted.

To better identify and monitor symptoms as efficiently as possible, it is important to evaluate which cognitive measures are sensitive enough, easily administered, and correlate well with objective deficits in cognitive function. In the cognitive aging literature, subjective cognitive impairment, or the patient's own appraisal of how their memory is functioning, has shown some success in adults with age-related cognitive disorders, such as mild cognitive impairment (MCI) and early stages of Alzheimer's disease (Gifford, Liu et al. 2015, Howieson, Mattek et al. 2015, Salem, Vogel et al. 2015, Yates, Clare et al. 2015), though not as reliably in cognitively normal older adults (Crane, Bogner et al. 2007, Buckley, Saling et al. 2015, Gifford, Liu et al. 2015). One study has shown that the Montreal Cognitive Assessment (MoCA) was able to detect cognitive impairments in aging HIV-positive veterans (Chartier, Crouch et al. 2015). Another study also recently showed that scores on the subjective Memory Functioning Questionnaire (MFQ) significantly correlated with an objective verbal memory performance in HIV-positive middle-aged adults, but not HIV-negative adults (Kamkwalala, Hulgan et al. 2017), suggesting that in cognitively vulnerable or impaired HIV-positive adults, comprehensive subjective measures of memory may be a salient and useful monitoring tool to identify those who may be developing objective cognitive impairments.

HIV-positive older adults may have to face the combined symptoms of HAND and risk of Alzheimer's Disease (Brew, Pemberton et al. 2005, Alisky 2007, Burt, Agan et al. 2008), while others may instead experience the combination of HAND and other neurodegenerative disorders, such as

Parkinson's Disease (Moulignier, Gueguen et al. 2015). These disease combinations may present additional complications of differing symptom profiles. One study evaluated deficit pattern differences between Older HIV positive adults, adults with Parkinson's, and adults with Alzheimer's, finding that while there was some symptom overlap between aging with HIV and each of these age-related disorders, aging with HIV is somewhere in between a "cortical" and "subcortical" cognitive disorder (Ciccarelli, Limiti et al. 2016)

Cognitive health and mental health have been shown to be closely linked, particularly in older adults. Somatic symptoms of depression and anxiety can contribute to poorer cognitive outcomes, and vice versa (Alexopoulos, Meyers et al. 2000, Nebes, Butters et al. 2000, Alexopoulos, Kiosses et al. 2005). The added complication of aging with HIV, a disorder with a well-documented high incidence of mood disorders, in particular major depression, poses a significant risk for worsening of cognitive outcomes compared to the HIV-negative population(Milanini, Catella et al. 2017). Not only are older persons more vulnerable to more persistent depressive symptoms despite treatment (Kalayam and Alexopoulos 1999, Lee, Potter et al. 2007, Steffens and Potter 2008, Wang, Krishnan et al. 2008), but depression in this older population is associated with cognitive impairments in and of itself: in the domains of executive functioning, processing speed, episodic memory, all of which are already impaired by the process of cognitive aging (Nebes, Butters et al. 2000, Lockwood, Alexopoulos et al. 2002, Morimoto, Gunning et al. 2012).

In HIV-positive adults, this higher incidence of cognitive and mood disorders (Dal-Bo, Manoel et al. 2015) can also exacerbate risk of substance abuse, relative to younger HIV-positive adults or older HIV-negative adults (Fumaz, Munoz-Moreno et al. 2012) and the combination of these factors may impact quality of life (Semple, Patterson et al. 1996, Moore, Fazeli et al. 2014). Recent studies have attributed the link between mood and cognitive symptoms to the function (and subsequently, dysfunction) of cholinergic pathways that are lost with age (Butters, Whyte et al. 2004, Crane, Bogner

et al. 2007, Iosifescu 2012). As discussed earlier, this may also be linked to the high incidence of nicotine use in the HIV population, as a way to ameliorate both cognitive and mood symptoms resulting from HIV-related cognitive deficits by exogenous activation of cholinergic circuits. At least one recent study even found that longitudinal nicotine administration (via transdermal patch) improved mood symptoms in a population of geriatric depressed patients (Gandelman, Kang et al. 2018), and through a similar mechanism, it may also be beneficial in this population. Treating mood symptoms without addressing cognitive impairments may be one reason that depression symptoms are less responsive to treatment in older and HIV-positive persons, and more consistent monitoring and treatment of both may lead to better outcomes.

In conclusion, this study showed that episodic memory performance was significantly different between HIV-negative and HIV-positive adults, and that in HIV positive adults, subjective memory scores correlate with objective measures of episodic recall and recognition memory. In addition, these differences in subjective memory and episodic memory performance were not explained by depressive symptoms. Memory impairments in HIV-positive adults may result from memory retention/retrieval failure, instead of encoding failure. This dataset may additionally indicate that the MFQ or similar comprehensive subjective memory impairment measures may have clinical value for consistent monitoring of cognitive changes in HIV-positive adults. Important future directions would be to identify which particular categories of subjective complaints may be most sensitive to objective memory impairments, as this would better inform what questions need to be asked about an individual's memory status to benefit both the patient's care and their clinician. Adding short subjective surveys of memory to routine clinical measurements may prove to be important to identify those who should be more closely monitored for the emergence of HAND symptoms, which may lead to earlier and possibly more effective interventions to improve the lives of adults aging with HIV.

CHAPTER VIII

STUDY II: CHOLINERGIC CORRELATES OF IMPAIRED COGNITIVE ABILITY IN HIV-ASSOCIATED NEUROCOGNITIVE DISORDERS

Rationale

The considerable overlap between symptoms of HIV-HAND and those of cognitive aging, may suggest a common underlying neuropathology to these symptoms experienced between older HIV-positive and HIV-negative adults. As discussed in Chapters 2 and 3, both HAND and abnormal cognitive aging feature deficits in memory (particularly, as I showed in Study One, episodic memory), attention, daily functioning, and executive control. However prior studies have shown that not only do these symptoms often emerge earlier in HIV-positive adults versus seronegative controls but they also may worsen more rapidly, causing more severe cognitive impairments than can be accounted for by age or education.

As these HIV-positive adults continue to survive into old age, understanding how best to identify and understand cognitive symptoms, particularly those that may interfere with necessary daily activities (remembering to take multiple medications, for example) may be a key factor in determining whether this population can continue to thrive in their old age, and reducing the risk of developing severe cognitive impairments of HAD as long as possible. Many prior studies have addressed parts of this question; the patterns of inflammation and neurodegeneration caused by persistent CNS infection (Vance 2004, Chang, Wong et al. 2008, Dawes, Suarez et al. 2008, Pfefferbaum, Rogosa et al. 2014, Wade, Valcour et al. 2015, Cole, Underwood et al. 2017), the influence of stable ART regimens on long-term cognitive status (Cohen, Boland et al. 2001, Robertson, Smurzynski et al. 2007, Winston, Puls et al. 2012, Crum-Cianflone, Moore et al. 2013, Heaton, Franklin et al. 2015), effects of CNS penetrance and potential toxicity of ART medications regarding cognitive status (Ciccarelli, Fabbiani et

al. 2011, Smurzynski, Wu et al. 2011, Treisman and Soudry 2016) salience of subjective vs. objective measures of cognitive ability (van Gorp, Satz et al. 1991), even the impact of lifestyle comorbidities such as smoking, drug and alcohol use, mood disorders on cognitive outcomes (Justice, McGinnis et al. 2004, Jernigan, Gamst et al. 2005, Wojna, Robles et al. 2007, Wright, Grund et al. 2010). One area that has not yet been extensively explored is the underlying neurotransmitter systems of cognition in the brains of adults living with HIV, and how certain regions may be particularly vulnerable to HIV-related damage.

Anti-Cholinergic Drug Challenge: A Cognitive "Stress Test"

In order to assess whether some of these deficits may result from damage to the brain's cholinergic system, the use of cholinergic antagonists can help to evaluate the relative contribution of cholinergic impairment to the symptoms of HAND; whether the cholinergic system is a primary target of the HIV virus, or if damage to the cholinergic system only accounts for a portion of the symptoms. Differential response to cholinergic blockade is a useful methodological probe to assess cholinergic tone, or the integrity of the cholinergic receptor system. As discussed in chapter 4, the nAChR and mAChR are both widely distributed throughout the brain, pre-and post-synaptically. Relative to a younger or more intact brain, a brain with fewer cholinergic cells and receptors is subsequently more sensitive to the effects of a similar dose of a receptor antagonist molecule, as a more significant percentage of the remaining receptors are more likely to bind the antagonist. With the use of an AChR antagonist like mecamylamine or scopolamine, a dose that may not have a meaningful impact on cognitive functioning in a healthy, unimpaired 35-year old, may be more likely to produce cognitive difficulties and performance changes in a 50-year old, or an individual experiencing some type of abnormal cognitive aging process, such as early signs of HIV-Associated Neurocognitive Disorder. Increased response to one antagonist or the other or the combination of both relative to placebo or compared to the HIV-negative individuals may indicate alteration of muscarinic and nicotinic receptor system function due to HIV serostatus, age, or a combination of the two.

With the antagonists mecamylamine and scopolamine, a heightened response to the effects of the receptor antagonists in the HIV-positive group, relative to the HIV-negative group, is a result of a baseline deficit in cholinergic system integrity. This study design does not directly show the relative loss of receptor density or integrity (as with an imaging study), however the results of this study will set important groundwork for further characterization of the status of acetylcholine receptor functioning in the HIV-positive brain, and how the HIV-virus itself interacts with these cell populations to produces the observed cognitive symptoms.

Identifying the cholinergic receptor system as a possible target of HIV related damage that leads to HAND also has therapeutic potential. In disorders such as ADHD (Potter, Ryan et al. 2009), schizophrenia (Griffith, O'Neill et al. 1998, Martin, Kem et al. 2004), and major depression (Newhouse, Sunderland et al. 1988, Salin-Pascual, Rosas et al. 1996, Lippiello, Beaver et al. 2008), identifying cognitive deficits attributed to the cholinergic system has created opportunities for cognitive treatments targeting these receptors. While cholinergic treatments may not necessarily alleviate the primary neuropathology underlying the disorder, it may be beneficial to alleviate some of the secondary cognitive impacts.

Similarly in the case of HIV/HAND, Though cholinergic nuclei are not the primary targets of the HIV virus, understanding alterations in cholinergic system functioning in an HIV-positive brain may be an important tool to improve cognitive status in aging HIV-positive individuals, and therefore it is important to investigate if there is a cholinergic component to the cognitive deficits experience in older individuals with HIV/HAND. Examining whether HIV-related damage to the cholinergic system is a part of the neurobiological sequelae that leads to HAND symptoms can potentially allow for future therapeutic strategies to improve cognition and minimize the differences in cognitive trajectory in the

HIV+ population, specifically by targeting this system, which can reliably be manipulated with known, or novel and future agents that are active at AChRs.

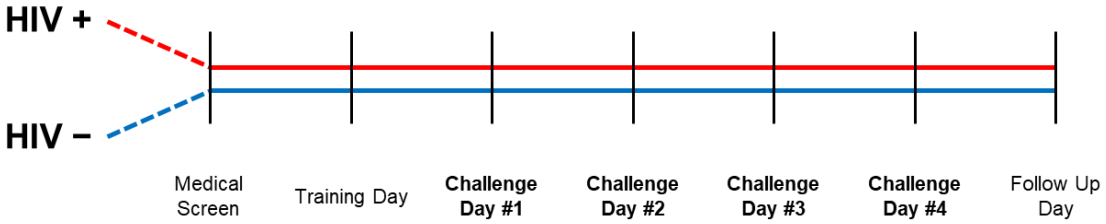


FIGURE 8: Overall Study Design and Timeline

Methods and Study Design

Study Participants

For this study, healthy HIV-positive and HIV-negative men and women over the age of 35 were considered for potential participation. All study procedures were approved and monitored by the Vanderbilt Institutional Review Board Human Research Protections Program. HIV-positive participants were recruited primarily from within the Vanderbilt University Medical Center Comprehensive Care Clinic participant population, with additional recruitment from the community HIV outreach program 'Nashville Cares'. HIV-negative participants were recruited primarily from the various clinics of the Vanderbilt University Medical Center, as well as the surrounding area of Middle Tennessee.

All participants were subjected to behavioral, cognitive, psychological and medical screening. Medical exclusions included: A current unmanaged Axis I or Axis II mood disorder, clinically significant cognitive impairment, impairment in liver, thyroid, or kidney functioning, abnormal electrocardiogram (ECG), or diagnosed severe cardiovascular disease within one (1) year prior, positive pregnancy test (if female) heavy nicotine use (determined via the Fagerström Nicotine Use Scale, if Item #4 ≥ 2, indicating 21 or more cigarettes per day) (Heatherton, Kozlowski et al. 1991), current alcohol abuse (≥ 6 drinks per day) or endorsement of active substance abuse (cocaine, heroin, methamphetamines, or barbiturates, particularly intravenously). Additional inclusion criteria in the HIV-positive group were:

participant must have a positive HIV diagnosis for at least five (5) years, and be at least six (6) months stable on an anti-viral medication regimen, with a viral load <50 copies, and CD4+ count of >200 at the time of screening.

After medical screen data was reviewed and approved by the investigator and medical monitor, participants were scheduled for a preliminary Training Day visit, to acclimate them to the environment of the Vanderbilt Clinical Research Center (CRC), as well as practice the four computerized tasks in the study battery (the Choice Reaction Time Task, the Verbal N-Back Task, the Spatial Selective Attention Task, and the Connors Continuous Performance Task) at least twice each, to establish stable individual performance prior to drug challenge days (see all task descriptions below). After participant arrival on the Training Day, personnel read detailed descriptions of each computerized task to the participant, and initiated practice versions of each task for the participant to ensure understanding of task instructions. Participants completed each practice task twice each.

Drug Procedures

For the purposes of this study, the low and high doses of Mecamylamine were 10mg and 20mg, and the low and high doses of Scopolamine were 2.5mcg/kg and 5mcg/kg, based on their measured weight at their Medical Screening Day. The doses of each drug were based on previous studies from our research group, and were sufficient to produce a significant response in young and old study participants without causing significant orthostatic hypotension (Mecamylamine) or delirium (Scopolamine) (Wesnes and Warburton 1984, Newhouse, Sunderland et al. 1988, Newhouse, Potter et al. 1992). While scopolamine is FDA approved for use in the over-the-counter transdermal patches (FDA, 2013) for the purposes of this study, use of scopolamine hydrobromide prepared for intravenous administration was approved by successful submission of an FDA Investigational New Drug Application (#126534),

Each participant was then randomized to receive each of four (4) possible study medications on each of their four (4) Challenge Days in a blinded order. All participants each received the following: 1. High-Dose Mecamylamine capsule + Placebo injection (MECA), 2. High-Dose Scopolamine injection + Placebo capsule (SCOP), 3. Low-dose Mecamylamine capsule + Low-dose Scopolamine injection (MECA+SCOP), 4. Placebo capsule + Placebo injection. (PLC). The PI created a 50x4 randomization table that was provided to Vanderbilt Investigational Drug Service Pharmacy (IDS), with each row having the numbers 1-4 ordered using a random number generator. IDS technicians assigned one of the four possible challenges to each number, and this assignment was blinded from the study investigator and nursing staff. Mecamylamine capsules and Scopolamine syringes were compounded and prepared for each participant by IDS technicians, who also oversaw the drug blinding for each participant during the course of the study. Capsule was administered at Time “0:00”, and injection was administered at Time “+30:00”, to allow for effect of either or both medications (if active) to peak at Time “+120:00”, when the cognitive testing battery began, based on prior evidence indicating that oral mecamylamine and intravenous scopolamine reach peak effectiveness after 120 and 90 minutes after administration respectively (Newhouse, Sunderland et al. 1988, Newhouse, Potter et al. 1994, Newhouse, Potter et al. 1994, Dumas, Saykin et al. 2008).

For each Challenge day, upon arrival each participant was administered a 3-point Standardized Field Sobriety Test (Stuster and Burns 1998) to assess horizontal gaze nystagmus (HGN), heel-to-toe Walk and Turn, and 30-second One-Leg Stand. Indicators of impairment (inability to smoothly track a horizontal object, loss of balance, swaying or hopping, missteps etc.) from each test are summed up into a Total Sobriety Score (TSS). This score was required to be <5 to proceed with the Challenge Day. This sobriety test was additionally administered at the conclusion of the challenge day, and this second TSS was required to be the less than or equal to the arrival score to ensure participant was safe to discharge. Due to known side effects of mecamylamine and scopolamine (dizziness, drowsiness, hypotension, dehydration) participants each were reclined at 45° in a CRC bed, with a normal saline

intravenous catheter drip at 125mL/hr. Participant vital signs were closely monitored, heart rate, blood pressure, temperature, respiratory rate and pupil size were recorded roughly every 30-60 minutes, as indicated in Figure 9.

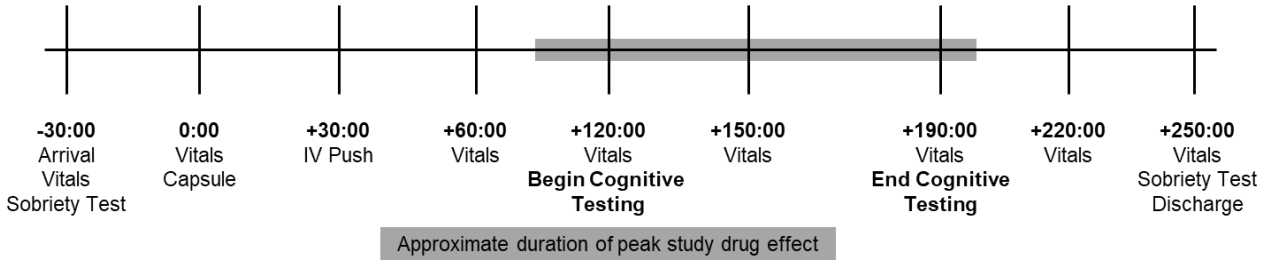


FIGURE 9: Study Challenge Day Timeline

Behavioral and Cognitive Battery

At the beginning and end of each challenge day, participants complete three (3) behavioral measures to assess alertness (Stanford Sleepiness Scale), mood disturbance (Profile of Mood States) and subjective mood state (Visual Analog Scales), and to ensure that any effects that the study medications may have had have resolved by the end of the day (Hoddes, Dement et al. 1972, Nyenhuis, Yamamoto et al. 1999, Reips and Funke 2008).

The Cognitive Battery was designed to assess the domains of cognition known to be impaired in HIV-Positive adults relative to seronegative controls, and incorporate measures that are sensitive to the effects of cholinergic antagonist medications. The entire study challenge battery, including chosen tasks, target cognitive domains and specific outcome measures for each task, are listed in Table 4. For each of the pen-and-paper tasks included (DSST, VF, Trails (B-A)) five (5) possible versions of the task were generated, to control for practice effects and ensure participants would never complete the same version of the task more than once under the different drug conditions. Task versions to be administered on each participant's challenge day were chosen via random number generator prior to participant arrival, excluding possible versions administered on previous challenge days.

Challenge Battery Task	Cognitive Domain(s)	Outcome Measures
Choice Reaction Time Task (CRT)	Visual Attention Processing Speed	Total Reaction Time Recognition Reaction Time Motor Reaction Time
Verbal Fluency Task (VF)	Verbal Retrieval	Total Words Generated (Three Letter Trials)
Spatial Selective Attention Task (SSAT)	Attention Executive Function	Validity Effect (Median Invalid Cue Reaction Time – Median Valid Cue Reaction Time)
Digit Symbol Substitution Task (DSST)	Processing Speed	Total Correct Symbols
Trails A and B Task (Trails B-A)	Psychomotor Speed Executive Function	Completion Time Δ (Trails B – Trails A)
Selective Reminding Task (SRT)	Episodic Memory	Total Mean Words Recalled (Eight Trials) Total Mean Consistency (Eight Trials) Total Mean Words Recalled – Delay Trial
Verbal N-Back (N-Back)	Episodic Memory	Match Condition d' 1-Back Condition d' 2-Back Condition d' 3-Back Condition d'
Grooved Pegboard	Psychomotor Speed	Completion Time Δ (Non-Dominant Hand – Dominant Hand)
Connors Continuous Performance (CPT)	Attention Inhibition	Omission Errors Commission Errors "Hit" Reaction Time "Hit" Reaction Time Standard Error Variability d' β Perseverations

TABLE 4: Summary of Study Cognitive Tasks, Domains, and Outcome Measures

The Choice Reaction Time Task (CRT) is a reaction time task where participants are instructed to wait and hold down their central “home” button, and wait for one of 6 target lights arranged in an arc to randomly illuminate. Once a trial begins and one of the arc lights turns on, the participant must release the home button, press the corresponding arc button, then return to hold the home button until the subsequent trial. Participants complete a total of 50 trials. Median total, recognition, and motor reaction times were calculated for each participant’s challenge performance across those 50 trials, then mean reaction times were calculated for each serostatus group for subsequent analyses. (Hindmarch 1984, Thorne, Genser et al. 1985, Newhouse, Potter et al. 1992).

The Spatial Selective Attention Task (SSAT) is a computerized spatial selective attention task to assess attentional shifting. Participants were instructed to use the left and right arrow buttons to indicate on which side of the screen the “target” image appears. They were also instructed to attend to the pre-trial “cue” in the center of the screen, which is programmed to incorrectly predict the target location in 20% of trials. Target variable for this task is the reaction time difference between correctly and incorrectly cued trials (Posner 1980).

The Trails A and B tasks (Reitan 1958, Tombaugh 2004) tests task switching, and psychomotor speed by having participants draw a continuous line through a randomly assorted array of numbers (Trails A) or letters and numbers (Trails B), in numerical/alphabetical order on an 8x11” page. The additional instruction for Trails B is to switch back and forth between letters and numbers (1, A, 2, B, 3, C etc.) also assesses executive functioning. Participants were given up to 120 seconds to complete Trails A, and up to 240 seconds to complete Trails B. Target outcome variable for this task is the difference (Δ) in completion time between tasks B and A (Trails B-A).

The Verbal Fluency Task (VF) assesses semantic retrieval, giving the participant 60 seconds to name as many words as they can, omitting proper nouns, that start with a given letter. Participants are

given three (3) trial letters on each challenge day, and see each set of letters only once. For the purposes of this study, five three-letter Verbal Fluency versions were generated of comparable letter difficulty and (Borkowski, Benton et al. 1967).

The Selective Reminding Task (SRT) (Buschke 1973) tests for encoding and retrieval of a verbal word list. Participants are read a 16-word list of high and low imagery words, one word per second, and are asked to recall as many words as they can over eight (8) trials. Between each trial, the experimenter re-reads only the words that were unsuccessfully recalled on the prior trial, and the participant must try again to recall all 16 on the next trial. Each word list was only used once per participant, and was randomly chosen before each challenge day. After a 20-minute delay (during which several other tests in the battery were performed), participants also completed a delayed recall trial, where they are asked to again recall all of the words from the list. Each of the 16 available lists were prepared to include a comparable variety of low and high imagery words, from a full set of 160 words.

The Grooved Pegboard Task (GP) (Ruff and Parker 1993) is a timed task to assess fine motor functioning of both the dominant and non-dominant hand. Participants were instructed to insert “key” pins into the board one at a time, in the correct orientation, while only manipulating the pins with one hand at a time. Completion time, (time to fill in all 25 pins), was recorded for each hand. Outcome variable for this task is the difference between non-dominant and dominant hand completion time.

The verbal N-Back task (Kirchner 1958, Kane, Conway et al. 2007) is a working memory task, with four conditions of increasing difficulty/attentional load. Participants see letters one per second on the computer screen and are asked to respond “Match” or “Mismatch” for each letter, according to the four task conditions: “Target Match”, “1-Back”, “2-Back”, and “3-Back”. In the “Target Match” condition, participants are first given a target letter, and when that letter appears in the sequence, they are

instructed to respond with the “Match” button, while all other letters are “Mismatch”. For the “1-Back”, “2-Back”, and “3-Back” conditions, participants are instructed to respond with the “Match” button if the letter that appears on the screen is the same as the letter that appeared in the sequence one, two, or three letters previously, and to respond to all other letters as “Mismatch”.

The Digit Symbol Substitution Task (DSST) (Gilmore, Royer et al. 1983, Salthouse 1992) assesses working memory and psychomotor speed. Participants are given a page with a key corresponding the numerals 1-9 with nine distinct symbols, and in the grid of 100 randomized numbers below, instructed to fill in as many of the correct symbols as possible within the allotted 120s.

Connors Continuous Performance Task (CPT) (Rosvold, Mirsky et al. 1956, Conners, Epstein et al. 2003) is an assessment of sustained attention, reaction time, executive control, and inhibition. Letters are presented on the screen one at a time, and participants are instructed to press the spacebar for every letter that appears except for the letter “X”, in which case they are instructed to withhold pressing the spacebar, and wait for the next letter to appear. Task is divided into three blocks of 1-second, 2-second, or 4-second intertrial intervals between letters, and each block is performed twice.

Statistical Analysis

Demographic variables (age, years of education, sex) and Memory Functioning Questionnaire (MFQ), Beck Depression Inventory (BDI), and International HIV Dementia Scale (IHDS) scores were compared between serostatus group by independent samples T-Test. In the HIV-positive group, individual CPE Score was derived from reported ART medication regimen at Medical Screening, as described by the CHARTER Group (Letendre, Ellis et al. 2010).

The overall statistical approach was to examine placebo challenge day performance for initial comparison of the HIV-negative and HIV-positive group’s performance on the cognitive battery, then

examine adjusted performance under the influence each of the active drug challenge days separately, by comparing change scores from their performance on the Placebo/Inactive challenge day. This approach evaluated the differences that could be explained by HIV-status, and which differences in performance can be attributed to the effects of the challenge day medications. Placebo challenge day performance scores and unadjusted cognitive battery performance scores were compared between serostatus groups by independent samples T-Tests. Repeated Measures mixed-model ANOVA was additionally performed across the three active drug challenge days, on all unadjusted cognitive outcome measures found to be significantly different on the Placebo/Inactive challenge day.

Performance change scores from placebo were derived for each performance measure by subtracting Placebo day cognitive performance scores from unadjusted performance scores on Mecamylamine, Scopolamine, and Meca + Scop challenge days to control for group differences in performance. Resulting change scores were compared across serostatus groups by independent samples T-Tests.

Mixed-model repeated measures two-way ANOVA was performed on unadjusted cognitive performance scores, and calculated change scores, on the unadjusted and change score outcome variables shown to be significantly different between the serostatus groups on the Placebo (Inactive) challenge day, to determine if there was an interaction between Drug Challenge and HIV-Status (Drug x HIV interaction). I also assessed the effect of age and BDI score as covariates on this interaction analysis.

To determine if the active drug challenges (Mecamylamine, Scopolamine, and Meca + Scop) had the intended physiological effects on participants, and to confirm that the active drugs had reached efficacious levels at the time of task battery initiation, unadjusted vitals measurements (Systolic/Diastolic BP, heart rate, respiratory rate, and pupil size) were compared within each group,

between the vitals timepoint at Time Zero (0:00) directly before they ingested the capsule, and the vitals timepoint immediately prior to the initiation of the challenge task battery (+120:00) under each active drug condition using paired samples T-Tests. Additionally, repeated measures mixed-model ANOVA was performed across the seven (7) vital signs timepoints after receiving the capsule (Time 0:00, +60:00, +120:00, +150:00, +190:00, +220:00, and +250:00) to evaluate changes in vitals and physiological effects of the study medications in each group over the course of the day under each of the four drug challenge days, and any significant analyses were further evaluated using Paired T-Tests.

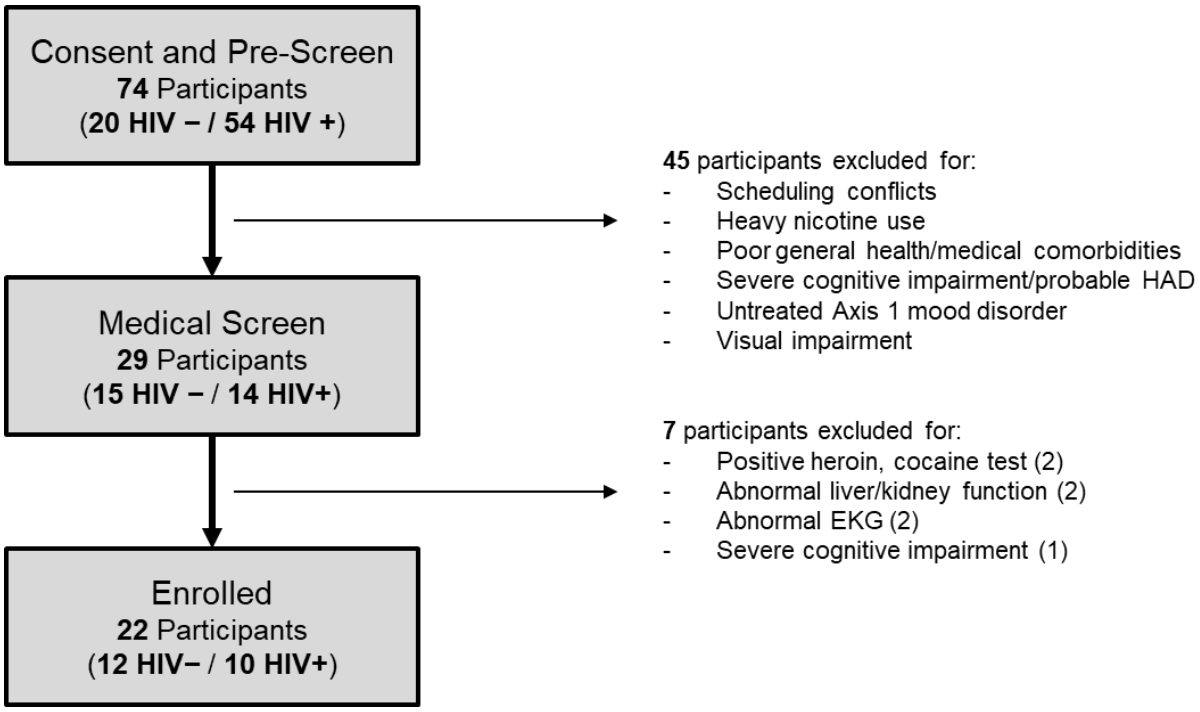


FIGURE 10: Consort Diagram for Study Participant Attrition

Results

A total of 74 participants (54 HIV-Positive/ 20 HIV-Negative) were pre-screened for participation, and gave written informed consent, approved by both the Vanderbilt University Institutional Review Board and Human Research Protections Program. After Pre-Screening, 45 participants were excluded, and 29 remaining participants (14 HIV-Positive/ 15 HIV-Negative) were cleared for Medical Screening.

After medical, behavioral, psychiatric, and cognitive screening, 7 further participants were excluded from participation: 2 tested positive for narcotics, 2 were excluded for abnormal kidney/liver function which medical monitor determined would interfere with their ability to metabolize the study challenge drugs, 2 participants were excluded for an abnormal EKG, and 1 was too severely cognitive impaired (Figure 10). The remaining 22 participants (10 HIV-Positive/ 12 HIV-) were enrolled to complete the study protocol. After enrollment, one HIV-positive participant was unable to complete the protocol due to inability to place an IV, and the final study N was 21 participants (10 HIV-Positive, 11 HIV-Negative).

		HIV-Negative	HIV-Positive	p
		(n=12)	(n=10)	
Sex	*Male*	3	8	0.008 *
	Female	7	2	
Age	*Range*	38-67	38-60	
	Mean	48.8	52.2	0.380
Race	*White*	6	5	
	Black	4	5	
Years of Education		15.3	14.1	0.198
Tobacco Use	*Yes*	1	4	
	No	8	5	
	Quit	1	1	
MFQ Score		312.0	293.3	0.305
BDI Score		3.9	11.4	0.001 *
IHDS Score		11.8	10.7	0.005 *
CPE		--	8.6	
TSI		--	18.0	
CD4+ Count	*Screen*		363.8	
	Nadir	--	340.7	
APOE Genotype	*E2/E3*	1	1	
	E3/E3	5	7	
	E3/E4	3	2	

TABLE 5: Baseline Characteristics and Screening Scores, by Serostatus Group

Descriptive Means for screening measures can be found in Table 5. Participant groups did not differ statistically by racial distribution or years of education. The HIV-positive group also had a higher number of participants with a history or current use of tobacco (in the above table, participants who had

used tobacco within the past two (2) years were considered current tobacco users, participants who had previously used tobacco but not within the last two (2) years were considered to have "Quit"). Chi-squared analysis showed significantly different sex distribution in each serostatus group ($\chi^2(1) = 7.103$, $p = 0.008$). Participant age was not statistically different between serostatus groups ($t(20) = -0.897$, $p = 0.380$). MFQ score was also not statistically different between groups ($t(18) = -1.274$, $p= 0.305$). APOE-E-4 genotyping showed that the majority of participants carried the E3/E3 genotype in both groups, and about the same number of carriers of the E4 risk factor allele in each group. Two participants in the HIV-negative groups had inconclusive genotyping tests. The HIV-positive group reported significantly higher mean BDI Score ($t(18) = -4.304$, $p< 0.001$) and IHDS Score ($t(18) = 2.616$), $p= 0.005$) compared to the HIV-negative group. HIV-related biological measures (CD4 Counts, Time Since Infection, Central Penetrance Effectiveness Score) are also reported in Table 5.

Physiological and Physical Effects of Challenge Agents

Primary side effects experienced by participants were consistent with known effects of the active challenge drugs. For Mecamylamine, effects included drowsiness, decreased blood pressure (particularly upon standing) and pupillary dilation. For Scopolamine, effects included dry mouth/dry eyes, and drowsiness. One participant reported side-effects significant enough to withdraw from the study: dry eyes persisting into the following day.

Placebo (Inactive) Drug Challenge

Vitals signs measurements (systolic/diastolic blood pressure, heart rate, respiratory rate and pupil dilation) on the Placebo Challenge day were generally not significantly different between groups at arrival (see table 6). Bilateral pupil size was slightly increased in the HIV-negative group at a trend level ($t(18) = 3.472$, $p=0.070$), but the differences between the groups was not clinically meaningful. Repeated measure mixed-models ANOVA analysis for the seven vitals timepoints showed a significant

main effect of timepoint on diastolic blood pressure and heart rate in both groups, likely attributed to spending the majority of challenge day reclined (Diastolic BP: ($F(6) = 3.168$, $p = 0.007$; Heart Rate: $F(6) = 7.775$, $p < 0.001$), as well as a trend-level interaction between timepoint and HIV-status on left pupil size ($F(6) = 1.847$, $p = 0.097$) (Table 7).

Participant cognitive battery performance during the placebo challenge day was significantly different between serostatus groups on a number of measures (Table 8). On the Choice Reaction Time Task, HIV-positive participants had significantly slower reaction times on the Total RT ($t(17 = -2.75$, $p = 0.014$) Recognition RT ($t(17) = -2.654$ $p = 0.017$) and Motor RT ($t(17) = -2.225$ $p = 0.04$) components of the task. HIV-positive participants also competed fewer total symbols on the DSST ($t(17) = 2.366$ $p = 0.03$), and correctly recalled fewer total words on the SRT ($t(17) = 2.629$ $p = 0.018$) compared to the HIV-negative group. No other cognitive measures were found to be significantly different between serostatus groups on the Placebo challenge day.

	HIV-Negative	HIV-Positive	p-value
Systolic BP	122.64 (12.65)	128.00 (15.52)	0.488
Diastolic BP	74.36 (8.33)	78.75 (9.38)	0.297
Heart Rate	74.27 (8.40)	73.00 (11.92)	0.787
Respiratory Rate	15.82 (2.60)	17.25 (1.83)	0.201
L. Pupil	3.09 (0.94)	2.38 (0.52)	0.070 †
R. Pupil	3.09 (0.94)	2.38 (0.52)	0.070 †

TABLE 6: Vital Signs *(Mean(SD))* by Serostatus Group at Arrival, Placebo/Inactive Challenge Day (†= p <0.100)

		Main Effect (Time)		Time x Group	
	df	**F**	**p-value**	**F**	**p-value**
Systolic BP	6	1.216	0.363	0.314	0.928
Diastolic BP	6	3.168	0.007 *	1.592	0.157
Heart Rate	6	7.750	<0.001 **	0.552	0.767
Respiration	6	1.271	0.277	1.140	0.342
L. Pupil	6	1.304	0.262	1.847	0.097 †
R. Pupil	6	1.266	0.280	1.532	0.175

TABLE 7: Repeated Measures ANOVA Table for Vital Signs, Placebo (Inactive) Challenge Day ((†= p <0.100, *= p <0.05, **= p <0.001)

		HIV-Negative	HIV-Positive	p-value
Choice Reaction Time Task	*Total RT*	664.36 (90.11)	819.75 (115.47)	0.014 *
	Recognition RT	393.47 (47.76)	438.50 (62.39)	0.017 *
	Motor RT	292.773 (62.44)	381.25 (110.55)	0.040 *
Verbal Fluency Task	*Total Word Score*	43.75 (13.06)	44.63 (11.53)	0.826
Digit Symbol Substitution Task	*Total Correct Symbols*	60.00 (16.72)	43.13 (14.45)	0.030 *
Trails Task	*Completion Δ (B-A)*	22.17 (21.54)	31.63 (51.03)	0.412
Grooved Pegboard Task	*Completion Δ (ND-D)*	4.58 (13.65)	11.25 (9.65)	0.338
Selective Reminding Task	*Total Words Recalled*	75.67 (16.85)	53.88 (20.36)	0.018 *
	Total Consistency	48.83 (5.98)	49.38 (4.07)	0.657
	Delayed Recall	8.67 (2.54)	6.50 (4.75)	0.189
Spatial Selective Attention Task	*Validity Effect (IV-V)*	2.91 (0.64)	3.12 (0.39)	0.421
N-Back Verbal Episodic Memory Task	*Match Condition d'*	3.12 (1.26)	2.04 (2.38)	0.217
	1-Back Condition d'	2.40 (1.25)	1.44 (1.37)	0.129
	2-Back Condition d'	1.49 (1.50)	0.43 (1.86)	0.184
	3-Back Condition d'	52.86(30.84)	37.63 (42.71)	0.392
Connors Continuous Performance Task	*Omission Errors*	0.83 (1.12)	1.88 (2.41)	0.180
	Commission Errors	8.17 (3.49)	8.75 (4.98)	0.742
	Hit RT	409.61 (34.34)	445.73 (63.81)	0.051 †
	Hit RT St. Err	5.13 (1.20)	5.81 (2.50)	0.439
	Variability	7.12 (4.43)	7.47 (3.62)	0.936
	d'	0.97 (0.36)	1.02 (0.50)	0.941
	β	0.57 (0.48)	0.81 (0.99)	0.451
	Perseverations	0.33 (0.65)	0.50 (0.75)	0.503

TABLE 8: Unadjusted Cognitive Scores *(Mean (SD))* by Serostatus Group, Placebo (Inactive) Challenge Day (†= p<0.100, *= p < 0.05)

Mecamylamine Drug Challenge

As previously described, participants were administered a capsule of 20 mg of mecamylamine orally approximately 120 minutes before initiating the cognitive challenge battery. Paired T-test analysis comparing vital signs at time 0:00 and the +120:00 time point showed significantly decreased systolic blood pressure measurements in the HIV-negative group (t(10) = 2.534, p= 0,03) but not the HIV-positive group (t(9) = 1.358, p= 0.212). There was also a trend-level difference in left and right pupil size with larger pupils in the HIV-negative group at the +120:00 timepoint (t(10) = -1.884, p= 0.089) Table 9A,B). No other vital signs measurements were significantly different between these time points in either group.

HIV-Negative			
	Time 0:00	**Time +120:00**	**p-value**
Systolic BP	125.27 (20.79)	115.82 (17.57)	0.030 *
Diastolic BP	72.18 (11.89)	69.45 (8.98)	0.372
Heart Rate	70.45 (6.85)	68.73 (7.43)	0.448
Respiratory Rate	17.27 (2.05)	17.18 (2.86)	0.902
L. Pupil	3.455 (0.65)	3.77 (0.68)	0.089 †
R. Pupil	3.455 (0.65)	3.77 (0.68)	0.089 †

HIV-Positive			
	Time 0:00	**Time +120:00**	**p-value**
Systolic BP	125.00 (18.85)	116.10 (10.98)	0.212
Diastolic BP	76.89 (7.753)	74.60 (8.09)	0.483
Heart Rate	69.00 (13.87)	74.00 (11.95)	0.11
Respiratory Rate	18.00 (1.73)	17.20 (1.93)	0.397
L. Pupil	2.722 (0.667)	3.05 (1.01)	0.282
R. Pupil	2.667 (0.71)	3.00 (1.05)	0.282

TABLE 9 A, B: Vital Signs *(Mean (SD))* Obtained Before Medication Administration, and Before Cognitive Testing, by Serostatus Group, Mecamylamine Challenge Day (†= p <0.100, *= p <0.05)

		Main Effect (Time)		Time x Group	
	df	F	p-value	F	p-value
Systolic	6	3.598	0.003 *	0.757	0.605
Diastolic	6	3.606	0.003 *	0.052	0.999
Heart Rate	6	5.603	<0.001 **	1.635	0.144
Respiration	6	1.585	0.159	1.166	0.330
L. Pupil	6	3.456	0.004 *	0.107	0.995
R. Pupil	6	3.344	0.005 *	0.190	0.979

TABLE 10: Repeated Measures ANCOVA Table for Vital Signs, Mecamylamine Challenge Day (*= p <0.05, **= p <0.001)

Repeated measure mixed-models ANOVA analysis for the seven vital signs timepoints showed a significant main effect of time on systolic and diastolic blood pressure, heart rate, and bilateral pupil size in both serostatus groups over the course of the mecamylamine challenge day, but no significant interaction between HIV-group and timepoint was seen on any vital sign measure (See table 10).

All cognitive change scores from the Placebo challenge day to the Mecamylamine challenge day can be found in Table 12, by serostatus group. On the CRT, Recognition reaction time change scores from placebo were significantly different between serostatus groups, with the HIV-positive group showing greater slowing ($t(17) = -3.273$, $p= 0.004$). Group difference between change scores for Total RT also approached significance, ($t(17) = -1.700$, $p = 0.107$, and again the HIV-positive group performed more slowly than the HIV-negative group, with respect to their placebo challenge day performance. On the Connors CPT, there was a significant group difference on the Beta (response style) variable, with the HIV-positive group having an increase in beta relative to placebo versus a decrease in the HIV-negative group ($t(17) = -2.509$, $p= 0.023$) after mecamylamine.

		HIV-Negative	HIV-Positive	p-value
Choice Reaction Time Task	*Total RT*	694.363 (87.42)	950.611 (178.05)	0.001 *
	Recognition RT	395.76 (39.94)	484.78 (64.68)	<0.001 **
	Motor RT	318.41 (65.01)	465.83 (159.64)	0.012 *
Verbal Fluency Task	*Total Word Score*	43.18 (14.18)	43.89 (14.79)	0.897
Digit Symbol Substitution Task	Total Correct Symbols	53.18 (19.32)	34.89 (17.58)	0.042 *
Trails Task	*Completion Δ (B-A)*	25.36 (17.69)	49.89 (41.79)	0.094 †
Grooved Pegboard Task	*Completion Δ (ND-D)*	5.55 (11.79)	1.44 (28.90)	0.672
Selective Reminding Task	*Total Words Recalled*	76.73 (16.13)	56.11 (24.90)	0.038 *
	Total Consistency	47.55 (4.92)	50.56 (5.18)	0.200
	Delayed Recall	8.73 (2.94)	5.00 (4.18)	0.031 *
Spatial Selective Attention Task	*Validity Effect (IV-V)*	45.15 (15.16)	28.69 (77.54)	0.497
N-Back Verbal Episodic Memory Task	*Match Condition d'*	2.98 (0.33)	3.08 (0.59)	0.619
	1-Back Condition d'	3.29 (1.26)	2.24 (1.25)	0.077 †
	2-Back Condition d'	1.83 (1.49)	1.17 (1.30)	0.308
	3-Back Condition d'	1.25 (1.46)	0.69 (1.14)	0.371
Connors Continuous Performance Task	*Omission Errors*	1.91 (1.81)	13.22 (19.63)	0.072 †
	Commission Errors	12.55 (6.20)	12.11 (6.84)	0.883
	Hit RT	418.40 (36.80)	489.76 (95.24)	0.034 *
	Hit RT St. Err	6.40 (2.45)	10.61 (6.85)	0.073 †
	Variability	10.7 (6.89)	20.24 (17.40)	0.113
	d'	0.77 (0.40)	0.79 (0.59)	0.949
	β	0.4436 (0.26)	1.75 (1.73)	0.023 *
	Perseverations	0.82 (1.47)	2.56 (2.74)	0.087 †

TABLE 11: Unadjusted Cognitive Scores *(Mean (SD))* by Serostatus Group, Mecamylamine Challenge Day (†= p < 0.100, *= p<0.05, **= p< 0.001

		HIV-Negative	HIV-Positive	p-value
Choice Reaction Time Task	*Total RT*	30.00 (41.30)	130.56 (192.20)	0.107
	Recognition RT	4.364 (21.21)	39.06 (24.92)	0.004 *
	Motor RT	25.636 (31.85)	31.071 (62.45)	0.809
Verbal Fluency Task	*Total Word Score*	-2.636 (9.38)	-1.37 (10.11)	0.783
Digit Symbol Substitution Task	*Total Correct Symbols*	-7.727 (12.23)	-5.125 (7.28)	0.600
Trails Task	*Completion Δ (B-A)*	7.455 (18.36)	13.750 (53.65)	0.720
Grooved Pegboard Task	*Completion Δ (ND-D)*	-0.909 (13.52)	-8.625 (32.51)	0.441
Selective Reminding Task	*Total Words Recalled*	0.909 (9.66)	-0.750 (7.11)	0.838
	Total Consistency	-0.727 (6.94)	0.875 (5.25)	0.591
	Delayed Recall	-0.091 (3.11)	-1.750 (2.12)	0.211
Spatial Selective Attention Task	*Validity Effect (IV-V)*	7.681 (30.91)	29.786 (60.37)	0.318
N-Back Verbal Episodic Memory Task	*Match Condition d'*	0.068 (0.75)	-0.046 (0.592)	0.725
	1-Back Condition d'	0.183 (0.657)	0.291 (1.54)	0.837
	2-Back Condition d'	-0.567 (0.91)	-0.111 (0.80)	0.273
	3-Back Condition d'	-0.249 (0.86)	0.401 (1.16)	0.179
Connors Continuous Performance Task	*Omission Errors*	1.182 (2.27)	5.88 (11.23)	0.191
	Commission Errors	4.454 (4.06)	1.625 (3.62)	0.135
	Hit RT	16.275 (30.08)	34.765 (36.19)	0.241
	Hit RT St. Err	1.281 (2.23)	3.151 (3.66)	0.184
	Variability	3.425 (6.76)	9.260 (14.08)	0.244
	d'	-0.229 (0.33)	-0.170 (0.49)	0.756
	β	-0.106 (0.46)	1.049 (1.44)	0.023 *
	Perseverations	0.454 (1.75)	1.500 (2.00)	0.243

TABLE 12: Cognitive Change Scores *(Mean (SD))* from Placebo, by Serostatus Group, Mecamylamine Challenge Day. (*= p<0.05)

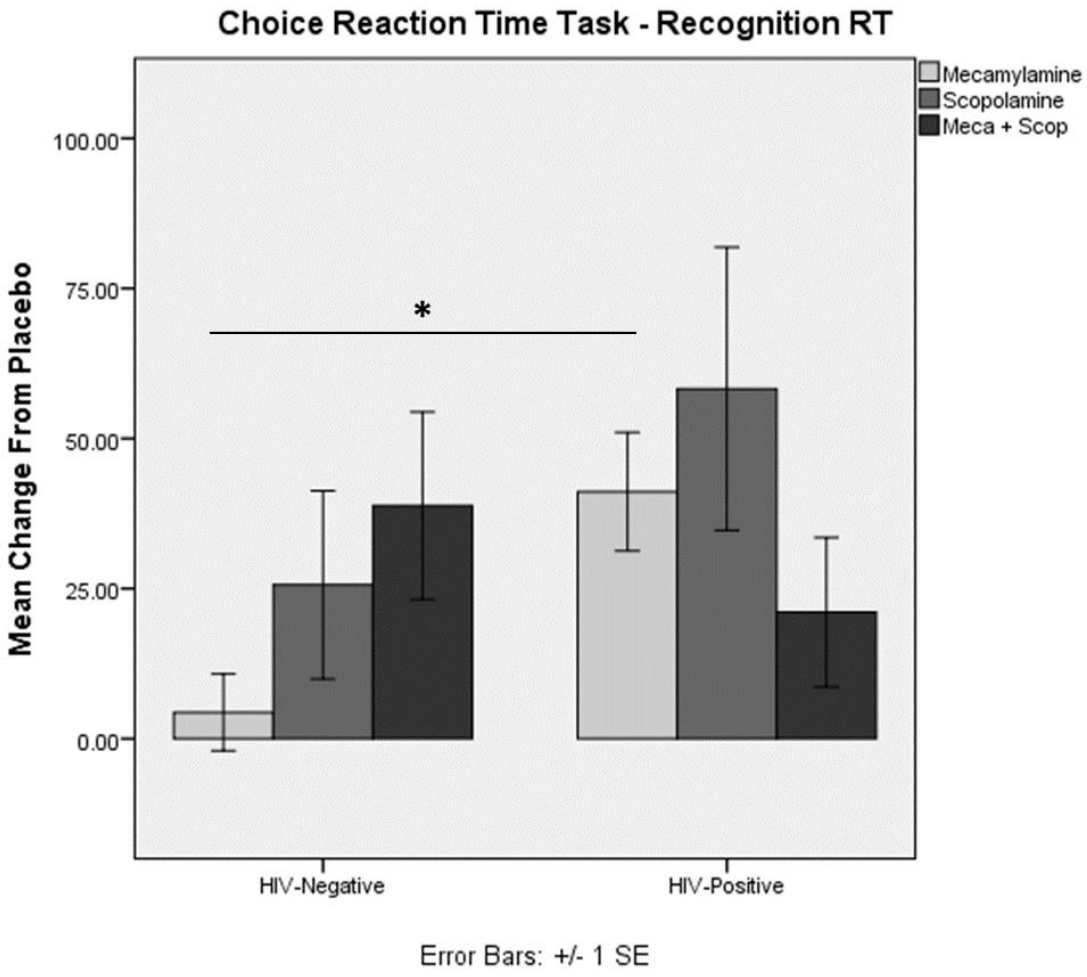


FIGURE 11: Change Scores from Placebo on the CRT Task – Recognition RT (*= p<0.05)

Scopolamine Active Drug Challenge

For the Scopolamine drug challenge day, participants were administered 5.0 mcg/kg of scopolamine intravenously (based on recorded weight at the Medical Screening Day), 90 minutes prior to the initiation of the challenge test battery. Paired T-test analysis comparing vital signs at Time 0:00 and the +120:00 time point showed a significant decrease in heart rate in the HIV-negative group ($t(10) = 2.722$, $p= 0.021$) but not the HIV-positive group ($t(6) = 0.713$, $p=0.503$) group, as well as a trend-level increase in bilateral pupil size in both the HIV-negative (both Left and Right: $t(10) = -1.929$, $p=0.083$) and HIV-positive (both Left and Right: $t(6) = -2.121$, $p= 0.078$) groups.

HIV-Negative			
	Time 0:00	Time +120:00	p-value
Systolic BP	121.45 (12.66)	120.64 (16.21)	0.843
Diastolic BP	73.18 (8.17)	71.00 (8.52)	0.297
Heart Rate	71.18 (11.01)	58.73 (11.67)	0.021 *
Respiratory Rate	16.55 (2.21)	16.55 (2.21)	1.000
L. Pupil	3.18 (1.25)	4.05 (1.46)	0.083 †
R. Pupil	3.18 (1.25)	4.05 (1.46)	0.083 †

HIV-Positive			
	Time 0:00	Time +120:00	p-value
Systolic BP	129.14 (20.94)	129.57 (24.14)	0.929
Diastolic BP	75.14 (6.19)	78.29 (8.18)	0.348
Heart Rate	64.00 (6.29)	60.71 (10.08)	0.503
Respiratory Rate	16.57 (1.90)	17.71 (2.43)	0.172
L. Pupil	2.57 (0.78)	3.00 (0.82)	0.078 †
R. Pupil	2.57 (0.78)	3.00 (0.82)	0.078 †

TABLE 13 A, B: Vital Signs *(Mean (SD))* Obtained Before Medication Administration, and Before Battery Initiation, by Serostatus Group, Scopolamine Challenge Day (†= p <0.100, *= p <0.05)

		Main Effect (Time)		Time x Group	
	df	F	p-value	F	p-value
Systolic	6	0.653	0.687	0.329	0.920
Diastolic	6	2.362	0.036 *	1.190	0.318
Heart Rate	6	6.622	<0.001 **	1.505	0.185
Respiration	6	0.381	0.889	0.654	0.686
L. Pupil	6	6.860	<0.001 **	0.252	0.952
R. Pupil	6	6.860	<0.001 **	0.265	0.952

TABLE 14: Repeated Measures ANOVA Table for Vital Signs, Scopolamine Challenge Day (*= p <0.05, **= p <0.001)

Paired comparisons of vital sign measurements between the 0:00 and +120:00 timepoints showed decreased HR in the HIV-negative group (t(10) 2.72, p= 0.0121) but not the HIV-positive group (t(6) = 0.713 p=0.503). Bilateral pupil size increased in both serostatus groups, and reached a trend level difference (HIV-Negative: t(10)= -1.929 p= 0.083; HIV-Positive: t(6)= -2.2, p= 0.078) (see table 13). Repeated measure mixed-models ANOVA analysis for the seven vital signs timepoints showed a significant main effect of timepoint on diastolic blood pressure, heart rate, and bilateral pupil size in both serostatus groups, but no significant interactions between time point and HIV-status (see table 14).

All change scores from the Placebo challenge day to the Scopolamine challenge day can be found in Table 16. On the CRT task, Total RT showed a trend-level difference between serostatus groups, change scores in the HIV-positive group were greater than the HIV-negative group ($t(16) = -1.88$, $p= 0.078$), suggesting a greater effect of the scopolamine in the HIV-positive group. On the SRT, the HIV-negative group had a significant decrease in words recalled compared to the placebo day, compared to little to no change in the HIV-positive group, and subsequently change scores were also significantly different between serostatus groups ($t(16) = -4.506$, $p < 0.001$) with a significantly larger change score in the HIV-negative group compared to the HIV-positive. On the CPT, change scores for commission errors were significantly different between serostatus groups, with a larger change score in the HIV-negative group ($t(16) = 2.466$, $p= 0.025$). There was also a trend level group difference in “Hit” reaction time on the CPT task, ($t(16) = -1.785$, $p= 0.093$), with a larger change in RT in the HIV-positive group.

		HIV-Negative	HIV-Positive	p-value
Choice Reaction Time Task	*Total RT*	735.409 (88.26)	964.50 (195.75)	0.004 *
	Recognition RT	425.70 (30.84)	481.86 (83.57)	0.005 *
	Motor RT	338.18 (74.69)	482.64 (163.76)	0.021 *
Verbal Fluency Task	*Total Word Score*	47.27 (15.32)	43.14 (16.47)	0.595
Digit Symbol Substitution Task	*Total Correct Symbols*	55.18 (17.34)	33.71 (8.01)	0.008 *
Trails Task	*Completion Δ (B-A)*	36.45 (31.39)	72.43 (40.11)	0.050 *
Grooved Pegboard Task	*Completion Δ (ND-D)*	9.18 (14.76)	10.57 (24.38)	0.881
Selective Reminding Task	*Total Words Recalled*	50.55 (15.69)	48.29 (16.98)	0.776
	Total Consistency	46.73 (3.49)	48.86 (6.69)	0.386
	Delayed Recall	5.00 (2.83)	4.86 (4.02)	0.303
Spatial Selective Attention Task	*Validity Effect (IV-V)*	30.81 (40.30)	21.50 (54.54)	0.693
N-Back Verbal Episodic Memory Task	*Match Condition d'*	2.57 (0.85)	2.12 (1.69)	0.464
	1-Back Condition d'	2.17 (1.78)	1.35 (1.39)	0.318
	2-Back Condition d'	1.74 (1.04)	0.94 (1.14)	0.149
	3-Back Condition d'	0.88 (1.37)	0.75 (1.70)	0.857
Connors Continuous Performance Task	*Omission Errors*	6.27 (8.26)	9.14 (9.81)	0.513
	Commission Errors	13.55 (5.50)	8.57 (5.32)	0.077
	Hit RT	429.08 (36.10)	500.17 (41.08)	0.001 **
	Hit RT St. Err	6.88 (1.99)	9.02 (4.83)	0.205
	Variability	10.78 (5.58)	15.49 (12.36)	0.284
	d'	0.75 (0.37)	1.07 (0.48)	0.129
	β	0.49 (0.49)	1.14 (1.17)	0.119
	Perseverations	1.27 (1.68)	1.43 (1.51)	0.845

TABLE 15: Unadjusted Cognitive Scores *(Mean (SD))* by Serostatus Group, Scopolamine Challenge Day (*= p<0.05, **= p< 0.001)

Subsequent to the large unadjusted difference and subsequent change scores shown on the SRT total words measure between the placebo challenge and the scopolamine challenge day, we additionally calculated percent change scores (using Placebo challenge day performance as 100%) for SRT Total words recalled on the three active drug challenge days, and performed Independent samples T-Tests on those values, and the percent change values were also significantly different between the serostatus groups -34.53% in the HIV negative group, and -13.37% in the HIV-positive group ($t(16) = 14.942$, $p < 0.001$).

		HIV-Negative	HIV-Positive	p-value
Choice Reaction Time Task	*Total RT*	71.05 (78.19)	187.29 (182.46)	0.078 †
	Recognition RT	25.636 (51.97)	58.28 (62.42)	0.246
	Motor RT	45.409 (38.07)	77.917 (62.44)	0.198
Verbal Fluency Task	*Total Word Score*	1.454 (8.08)	-1.143 (9.99)	0.552
Digit Symbol Substitution Task	*Total Correct Symbols*	-5.727 (7.94)	-11.714 (14.16)	0.264
Trails Task	*Completion Δ (B-A)*	18.545 (32.77)	47.571 (62.169)	0.211
Grooved Pegboard Task	*Completion Δ (ND-D)*	3.545 (15.31)	1.714 (28.82)	0.862
Selective Reminding Task	*Total Words Recalled*	-26.090 (9.94)	-7.71 (4.99)	<0.001 **
	Total Consistency	-1.545 (5.61)	-0.857 (5.43)	0.801
	Delayed Recall	-3.818 (2.82)	-2.142 (1.34)	0.164
Spatial Selective Attention Task	*Validity Effect (IV-V)*	22.045 (20.49)	29.250 (37.82)	0.674
N-Back Verbal Episodic Memory Task	*Match Condition d'*	-0.342 (1.27)	-1.057 (1.69)	0.319
	1-Back Condition d'	-0.949 (1.05)	-0.809 (1.81)	0.836
	2-Back Condition d'	-0.665 (0.90)	-0.492 (1.20)	0.733
	3-Back Condition d'	-0.610 (1.44)	-0.198 (1.21)	0.237
Connors Continuous Performance Task	*Omission Errors*	5.545 (7.95)	8.000 (9.78)	0.567
	Commission Errors	5.455 (4.18)	0.571 (4.18)	0.025 *
	Hit RT	26.949 (36.19)	59.19 (49.94)	0.093 †
	Hit RT St. Err	1.756 (2.13)	3.914 (4.86)	0.210
	Variability	3.479 (8.04)	9.086 (11.81)	0.246
	d'	-0.254 (0.34)	-0.014 (0.28)	0.143
	β	0.364 (0.57)	-0.055 (0.68)	0.463
	Perseverations	0.909 (1.22)	1.145 (2.62)	0.736

TABLE 16: Cognitive Change Scores *(Mean (SD))* from Placebo, by Serostatus Group, Scopolamine Challenge Day (†= p <0.100, *= p <0.05)

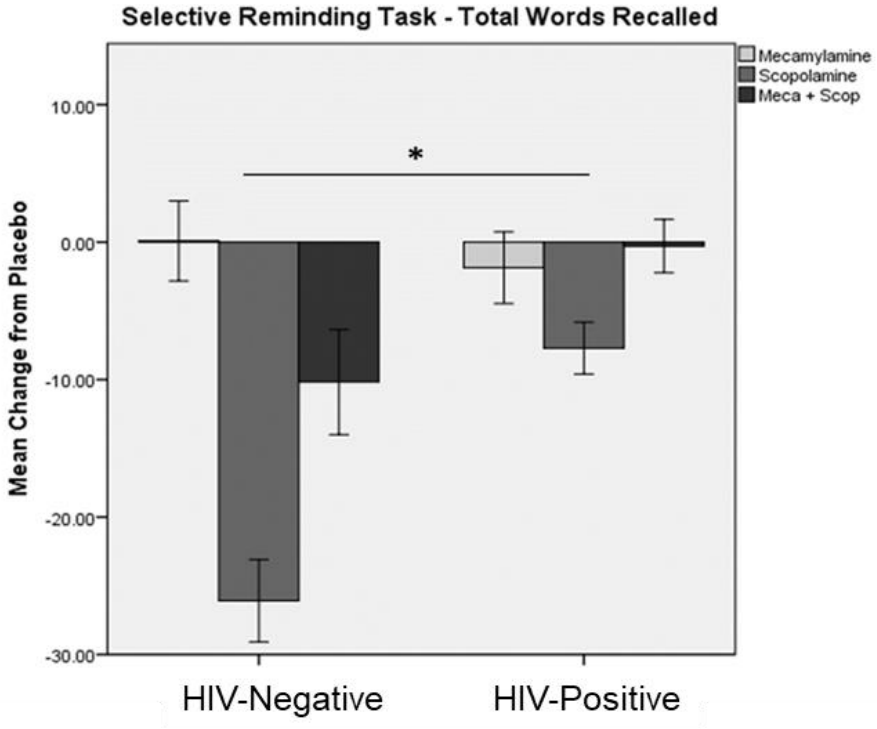


FIGURE 12: Change Scores from Placebo on the SRT, Total Words Recalled (* = p< 0.05)

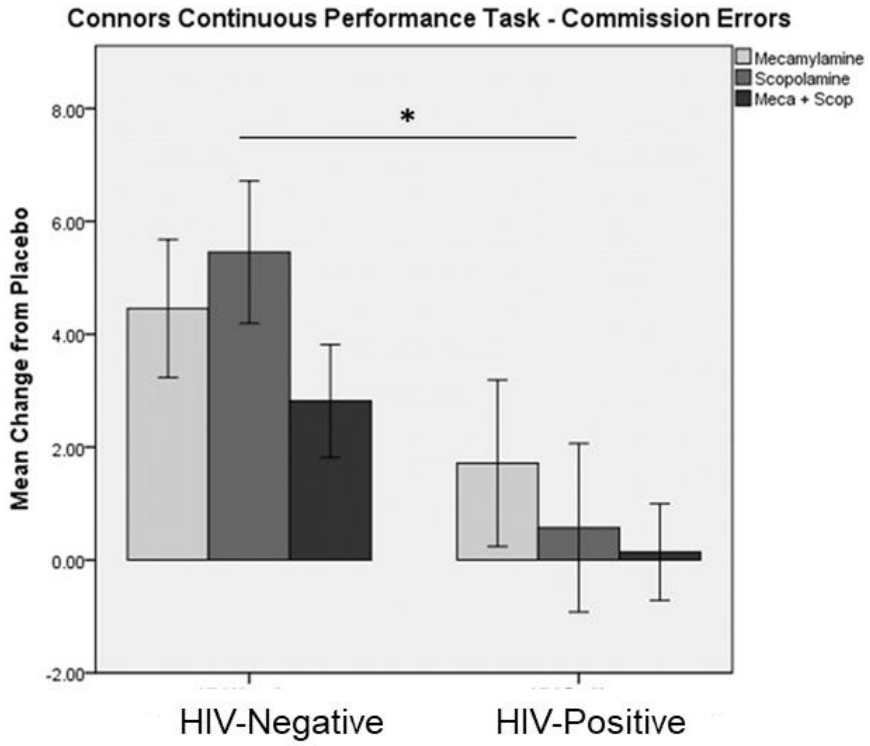


FIGURE 13: Change Scores from Placebo on the Connors CPT, Commission Errors (*= p<0.05)

Meca + Scop Active Drug Challenge

For the Meca + Scop challenge day, participants were administered a 10 mg capsule of mecamylamine orally approximately 120 minutes prior to task battery initiation, and an intravenous push of 2.5 mcg/kg scopolamine (based on recorded weight at Medical Screening Day) approximately 90 minutes prior to task battery initiation. Repeated measure mixed-models ANOVA analysis for the seven vital signs timepoints showed a significant timepoint x HIV status interactions for heart rate ($F(6) = 2.462$, $p= 0.029$) and respiration rate ($F(6) = 2.279$, $p = 0.041$) (Table 18), and a significant main effect of timepoint on systolic and diastolic blood pressure, heart rate, and bilateral pupil size over the course of the Meca + Scop challenge day.

All cognitive battery change scores from Placebo to the Meca + Scop challenge day can be found in Table 20. Change scores on the Trails (B-A) task were significantly higher in the HIV-positive group ($t(17) = -2.518$, $p=0.022$) indicating a greater effect of the Meca + Scop compared to the HIV-negative group. There was also a trend-level difference in performance change from placebo on the SRT total words recalled measure, with a larger difference in the number of words recalled in the HIV-negative group relative to placebo day performance ($t(17) = -2.518$, $p= 0.076$) than in the HIV-positive group, which performed relatively similarly between the Placebo and Meca + Scop challenge days ($t(17) = -1.890$, $p= 0.076$).

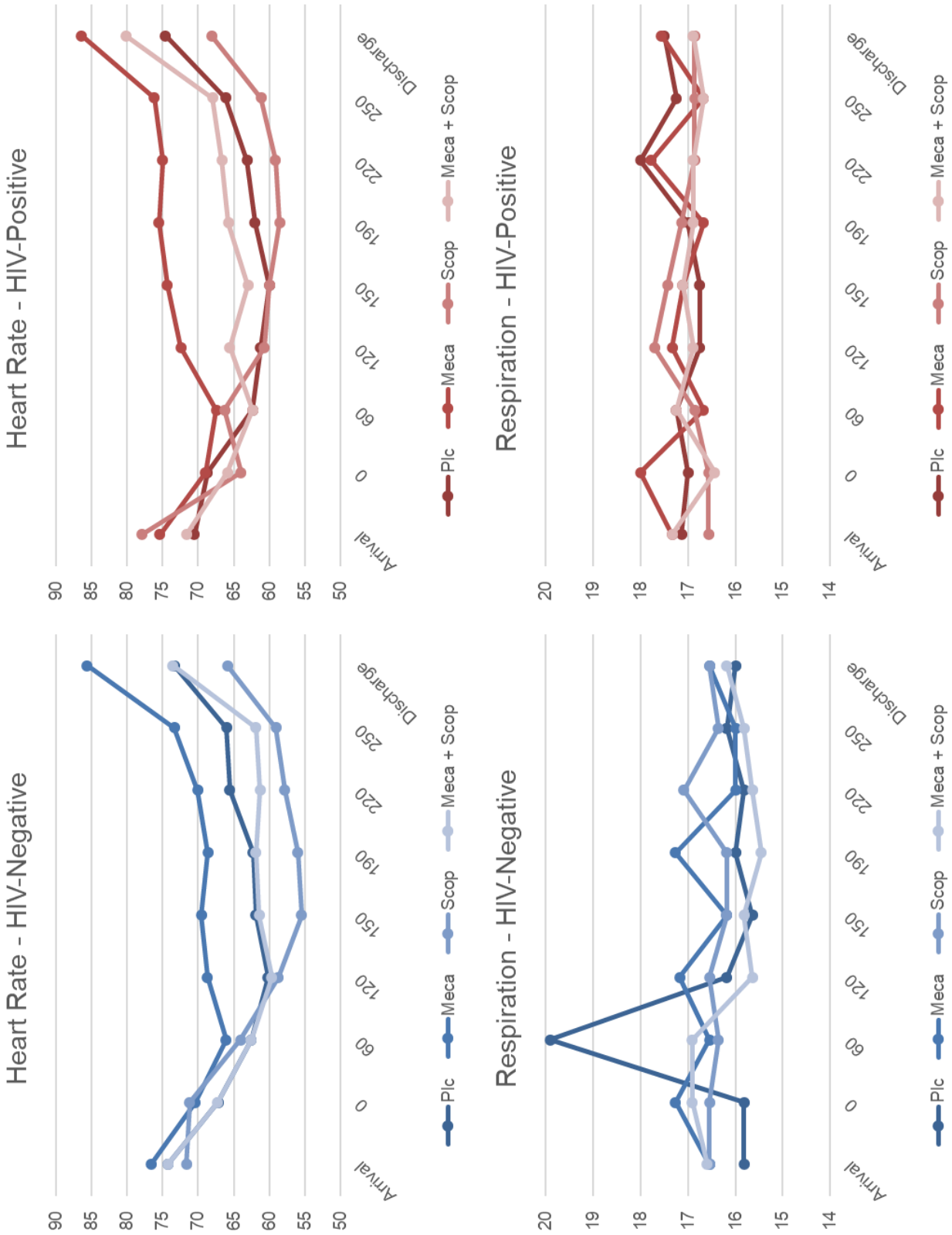


FIGURE 14, A, B: Mean Heart Rate and Respiration by Timepoint, Serostatus Group

HIV-Negative			
	Time 0:00	Time +120:00	p-value
Systolic BP	115.82 (10.92)	109.36 (13.71)	0.002 *
Diastolic BP	69.55 (6.73)	63.91 (6.30)	0.004 *
Heart Rate	67.27 (8.28)	59.64 (9.69)	0.004 *
Respiratory Rate	16.91 (1.375)	15.64 (1.50)	0.002 *
L. Pupil	3.00 (0.63)	3.45 (0.79)	0.01 *
R. Pupil	3.00 (0.63)	3.45 (0.79)	0.01 *

HIV-Positive			
	Arrival	Time +120:00	p-value
Systolic BP	127.89 (15.25)	124.22 (20.24)	0.498
Diastolic BP	74.44 (9.66)	75.56 (6.88)	0.817
Heart Rate	65.89 (8.41)	65.56 (13.03)	0.908
Respiratory Rate	16.44 (1.67)	16.89 (3.33)	0.594
L. Pupil	2.83 (0.50)	3.33 (0.87)	0.067 †
R. Pupil	2.94 (0.63)	3.33 (0.87)	0.043 *

TABLE 17 A, B: Vital Signs *(Mean (SD))* Obtained Before Medication Administration, and Before Cognitive Testing, by Serostatus Group, Meca + Scop Challenge Day (†= p <0.100, *= p <0.05)

		Main Effect (Time)		Time x Group	
	df	F	p-value	F	p-value
Systolic	6	2.520	0.025 *	0.785	0.584
Diastolic	6	2.990	0.010 *	1.043	0.402
Heart Rate	6	4.892	<0.001 **	2.462	0.029 *
Respiration	6	0.884	0.509	2.279	0.041 *
L. Pupil	6	3.979	0.001 *	0.262	0.953
R. Pupil	6	3.341	0.005 *	0.405	0.874

TABLE 18: Repeated Measures ANOVA Table for Vital Signs, Scopolamine Challenge Day (*= p <0.05, **= p <0.001)

		HIV-Negative	HIV-Positive	p-value
Choice Reaction Time Task	*Total RT*	760.41 (111.88)	946.61 (183.11)	0.012 *
	Recognition RT	433.03 (39.20)	472.72 (81.75)	0.034 *
	Motor RT	350.00 (89.11)	473.89 (125.46)	0.019 *
Verbal Fluency Task	*Total Word Score*	42.91 (15.32)	46.56 (13.23)	0.581
Digit Symbol Substitution Task	*Total Correct Symbols*	54.91 (15.97)	37.44 (14.61)	0.021 *
Trails Task	*Completion Δ (B-A)*	23.82 (15.66)	73.89 (42.56)	0.002 *
Grooved Pegboard Task	*Completion Δ (ND-D)*	9.45 (9.78)	7.33 (14.76)	0.704
Selective Reminding Task	*Total Words Recalled*	66.45 (16.11)	55.00 (21.94)	0.195
	Total Consistency	46.82 (4.24)	50.11 (4.94)	0.126
	Delayed Recall	6.00 (2.97)	5.56 (4.98)	0.807
Spatial Selective Attention Task	*Validity Effect (IV-V)*	35.41 (46.67)	48.56 (58.29)	0.592
N-Back Verbal Episodic Memory Task	*Match Condition d'*	2.5 (0.74)	2.63 (0.89)	0.715
	1-Back Condition d'	3.12 (1.11)	2.28 (1.80)	0.223
	2-Back Condition d'	2.31 (1.18)	1.76 (1.89)	0.432
	3-Back Condition d'	1.43 (1.25)	1.02 (2.08)	0.586
Connors Continuous Performance Task	*Omission Errors*	7.36 (13.87)	13.67 (26.39)	0.501
	Commission Errors	10.91 (5.01)	11.00 (6.98)	0.973
	Hit RT	439.36 (49.80)	505.99 (93.10)	0.550
	Hit RT St. Err	8.20 (5.39)	11.69 (10.03)	0.333
	Variability	14.44 (15.88)	20.05 (20.97)	0.505
	d'	0.77 (0.33)	0.88 (0.55)	0.586
	β	0.91 (0.67)	1.43 (1.67)	0.359
	Perseverations	1.82 (2.79)	4.00 (5.22)	0.247

TABLE 19: Unadjusted Cognitive Scores *(Mean (SD))* by Serostatus Group, Meca + Scop Challenge Day (*= p<0.05)

		HIV-Negative	HIV-Positive	p-value
Choice Reaction Time Task	*Total RT*	96.05 (92.04)	105.94 (110.86)	0.834
	Recognition RT	38.818 (51.75)	22.625 (30.79)	0.443
	Motor RT	57.227 (59.75)	51.071 (62.47)	0.837
Verbal Fluency Task	*Total Word Score*	-2.909 (12.57)	1.625 (10.568)	0.419
Digit Symbol Substitution Task	*Total Correct Symbols*	-6.00 (10.16)	-3.00 (5.32)	0.458
Trails Task	*Completion Δ (B-A)*	5.909 (14.747)	37.250 (37.84)	0.022 *
Grooved Pegboard Task	*Completion Δ (ND-D)*	3.818 (14.01)	-3.00 (16.61)	0.346
Selective Reminding Task	*Total Words Recalled*	-10.181 (12.69)	-1.125 (5.303)	0.076 †
	Total Consistency	-1.454 (7.21)	1.37 (6.91)	0.402
	Delayed Recall	-2.818 (3.22)	-1.125 (1.81)	0.199
Spatial Selective Attention Task	*Validity Effect (IV-V)*	17.454 (48.59)	10.786 (37.58)	0.762
N-Back Verbal Episodic Memory Task	*Match Condition d'*	-0.410 (0.69)	-0.319 (0.85)	0.799
	1-Back Condition d'	0.001 (1.46)	0.522 (2.57)	0.581
	2-Back Condition d'	-0.085 (1.64)	0.710 (1.78)	0.329
	3-Back Condition d'	-0.629 (1.79)	1.001 (2.50)	0.292
Connors Continuous Performance Task	*Omission Errors*	6.636 (13.87)	3.375 (6.12)	0.544
	Commission Errors	2.818 (3.31)	0.875 (2.95)	0.204
	Hit RT	37.229 (42.71)	40.789 (38.56)	0.854
	Hit RT St. Err	3.077 (5.61)	3.124 (3.89)	0.984
	Variability	7.134 (17.35)	6.690 (9.06)	0.948
	d'	-0.230 (0.19)	-0.054 (0.276)	0.122
	β	-0.055 (0.68)	0.349 (1.10)	0.347
	Perseverations	1.454 (2.62)	3.125 (5.06)	0.359

TABLE 20: Cognitive Change Scores *(Mean (SD))* from Placebo, by Serostatus Group, Meca + Scop Challenge Day (†= p <0.100, *= p<0.05)

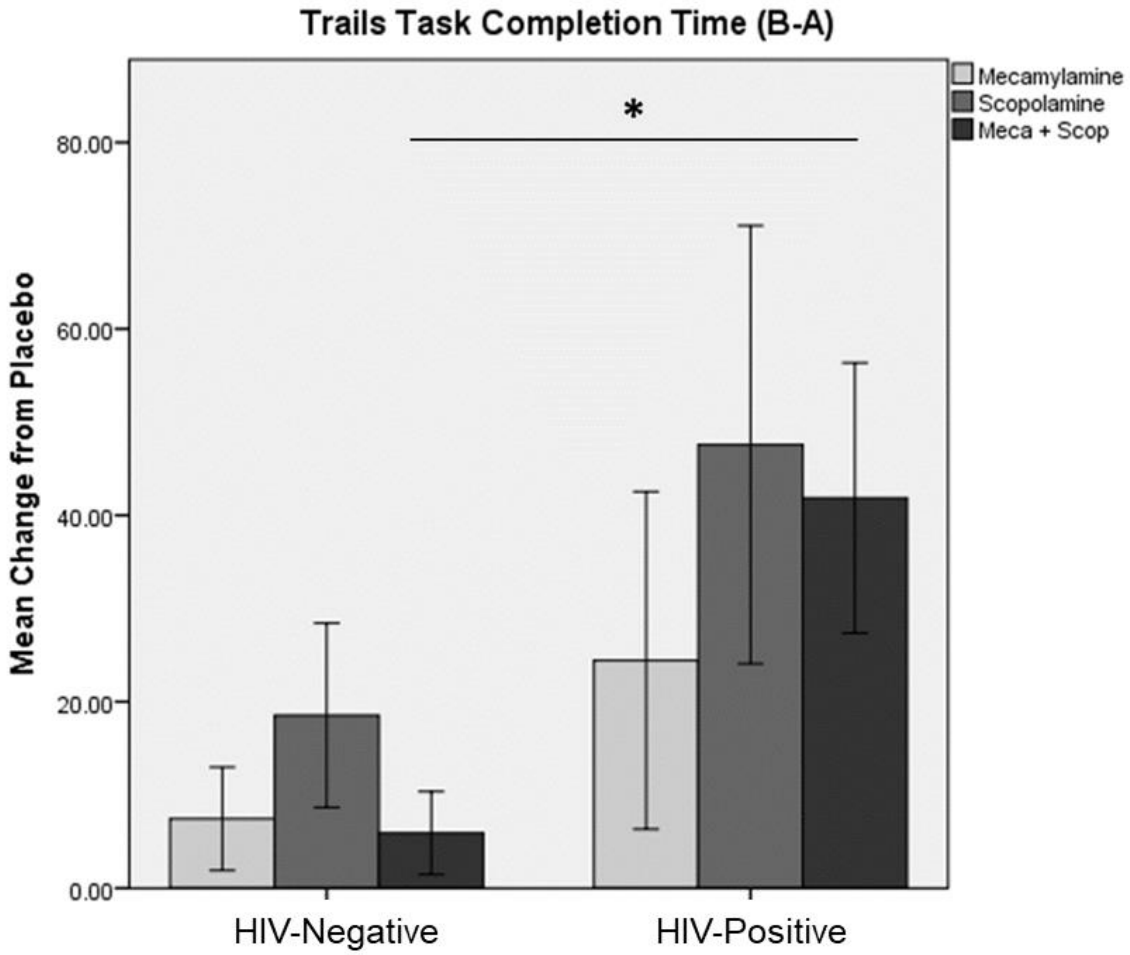


FIGURE 15: Change Scores from Placebo on the Trails Task, Completion Time Δ (*= p<0.05)

Examination of Overall Drug by Group Interactions and the Effect of Age, BDI Score

To better understand the relative effect of specific or general cholinergic blockade by serostatus group (Drug x HIV status interaction) on cognitive performance, a mixed model repeated measures ANOVA was performed on the unadjusted cognitive scores, and the change scores from the placebo challenge day and the active challenge days for each of the outcome measures that were significantly different between the serostatus groups on the placebo challenge day: the Trails (B-A), DSST, CRT, and SRT Total Words Recalled. Subsequently, repeated measures ANCOVAs were performed with age as a covariate, on the unadjusted and change score outcome measures. As referenced in earlier chapters, mood symptoms are more prevalent in the HIV-positive population (Justice, McGinnis et al. 2004, Au, Cheng et al. 2008), and have been associated with cognitive impairments (Kamkwalala,

Hulgan et al. 2017). An additional repeated measures ANCOVA was performed on unadjusted and change score measures with BDI score as a covariate for the Trails (B-A), DSST, CRT and SRT Total Words Recalled. All adjusted means for the Age and BDI covariate analyses are found in Appendix B.

When examining the unadjusted performance data for the three active drug challenges, I found a significant main effect of drug for all three measures of the CRT, as well as a significant drug x HIV-status interaction term for the Total RT and Recognition RT measures, but not the Motor RT measure. There were also significant main effects of the study drug for the DSST, Trails (B-A), and SRT Total unadjusted measures, but the drug x HIV-status interaction was only significant for the SRT (Table 21).

			Main Effect (Drug)		Drug x Group	
		df	F	p	F	p
Choice Reaction Time Task	*Total RT*	3	11.04	<0.001 **	3.339	0.021 *
	Recognition RT	3	5.972	0.002 *	3.299	0.028 *
	Motor RT	3	7.779	<0.001 **	2.149	0.106
Digit Symbol Substitution Task	*Total Correct Symbols*	3	3.740	0.017 *	0.995	0.422
Trails (B-A)	*Completion Δ (B-A)*	3	4.398	0.008 *	1.338	0.258
Selective Reminding Task	*Total Words Recalled*	3	17.022	<0.001 **	6.422	0.001 *

TABLE 21: Repeated Measures Mixed Model ANOVA Table, Unadjusted Cognitive Scores (*= p <0.05, ** p < 0.001)

			Main Effect (Drug)		Drug x Group	
		df	F	p	F	p
Choice Reaction Time Task	*Total RT*	2	2.097	0.139	4.986	0.013 *
	Recognition RT	2	1.954	0.158	4.780	0.016 *
	Motor RT	2	0.743	0.484	1.713	0.197
Digit Symbol Substitution Task	*Total Correct Symbols*	2	0.930	0.405	1.132	0.303
Trails (B-A)	*Completion Δ (B-A)*	2	1.423	0.256	0.447	0.643
Selective Reminding Task	*Total Words Recalled*	2	14.939	<0.001 **	5.672	0.008 *

TABLE 22: Repeated Measures Mixed Model ANOVA Table, Change Scores (*= p <0.05, ** p < 0.001)

When examining change score data, ANOVA analysis showed there were no significant main effects on any change score outcome variables, the drug x HIV status interaction terms were significant for the CRT Total RT Change Score ($F(2) = 4.986$, $p= 0.013$) and the CRT Recognition RT ($F(2) = 4.780$, $p= 0.016$) but not for the CRT Motor RT ($F(2) = 1.713$, $p= 0.197$) (see Table 22). The drug x HIV Status interaction term was also significant for SRT change score ($F(2) = 5.672$, $p= 0.011$). The change score ANOVA interaction term was again not significant for the DSST ($F(2) = 1.132$, $p= 0.303$) or the Trails (B-A) ($F(2) = 0.447$, $p= 0.643$) (see Table 22). Post-hoc pairwise comparisons analysis (with Tukey's HSD correction) for the three significant interaction terms determined that the Mecamylamine and Scopolamine challenge change scores were the most significantly different for the CRT Total interaction term ($p = 0.026$), and reached trend-level difference for the CRT Recognition interaction term ($p= 0.070$). On the SRT, the total words recall mean change scores on the Scopolamine challenge day was significantly different from both the Mecamylamine ($p< 0.001$) and Meca + Scop ($p = 0.002$) challenges.

For the unadjusted data analyses, Mixed Model Repeated Measures ANCOVA results showed that the age-adjusted drug x HIV-status interaction term was significant for the CRT Total RT ($F(3) = 3.743$, $p= 0.017$) and Recognition RT ($F(3) = 4.200$, $p= 0.011$) measures, and as well the SRT Total Words recalled score ($F(3) = 4.646$, $p= 0.007$) (see Table 23). When analyzing change scores (Table 24), there was a significant age-adjusted interaction term on the CRT Total RT change score ($F(2) = 7.429$, $p= 0.002$), CRT Recognition RT ($F(2) = 6.852$, $p= 0.004$), and SRT Total Words Recalled change scores ($F(2) = 3.938$, $p= 0.30$) . No other age-adjusted main effects or interaction terms reached statistical significance. Post-hoc pairwise comparisons (with Tukey's HSD correction) again found that age-adjusted mean change scores were most significantly different between the Mecamylamine and Scopolamine challenges for the CRT Total RT ($p= 0.029$) as well as a trend level difference for the CRT Recognition RT ($p = 0.096$). on the SRT Total words recalled interaction term,

the scopolamine challenge day age-adjusted change scores were again significantly different from both Mecamylamine ($p < 0.001$) and Meca + Scop ($p= 0.002$).

			Main Effect (Drug)		Drug x Group	
		df	F	p	F	p
Choice Reaction Time Task	*Total RT*	3	0.873	0.461	3.743	0.017 *
	Recognition RT	3	1.300	0.286	4.200	0.011 *
	Motor RT	3	0.435	0.729	2.042	0.121
Digit Symbol Substitution Task	*Total Correct Symbols*	3	1.585	0.206	1.399	0.255
Trails (B-A)	*Completion Δ (B-A)*	3	0.842	0.478	1.031	0.388
Selective Reminding Task	*Total Words Recalled*	3	0.695	0.560	4.646	0.007 *

TABLE 23: Repeated Measures Mixed Model ANCOVA Table (incl. Age as Covariate), Unadjusted Cognitive Scores (*= p <0.05, ** p < 0.001)

			Main Effect (Drug)		Drug x Group	
		df	F	p	F	p
Choice Reaction Time Task	*Total RT*	2	2.402	0.108	7.492	0.002 *
	Recognition RT	2	1.969	0.157	6.852	0.004 *
	Motor RT	2	0.853	0.436	2.363	0.111
Digit Symbol Substitution Task	*Total Correct Symbols*	2	1.045	0.364	1.537	0.231
Trails (B-A)	*Completion Δ (B-A)*	2	1.098	0.347	0.497	0.613
Selective Reminding Task	*Total Words Recalled*	2	0.794	0.461	3.938	0.030 *

TABLE 24: Repeated Measures Mixed Model ANCOVA Table (incl. Age as Covariate), Change Scores (*= p <0.05, ** p < 0.001)

When BDI was added as a covariate to the unadjusted data analyses, several of the target measures had significant or trend-level main effects of the study medications, and significant interaction terms (see Table 26). On the CRT, after adjusting for BDI score there was a trend-level drug x HIV status interaction term for the Motor RT variable ($F(3) = 2.599$, $p = 0.064$) and a significant interaction term for the Recognition RT variable ($F(3) = 3.943$, $p = 0.023$). While there were trend-level main effects on the CRT Motor RT and Trails (B-A) variables, the BDI-score adjusted interaction was not statistically significant. On the SRT, there was a significant interaction term for the Total Words Recalled variable ($F(3) = 4.309$, $p= 0.009$).

When analyzing BDI-adjusted change scores, the results were similar to the analyses on the unadjusted scores. The only significant main effect of drug was on the SRT Total Words Recalled, and there was also a significant BDI-adjusted drug x HIV status interaction term for this variable ($F(3) = 3.955$, $p = 0.030$). There was also a trend-level interaction term for the CRT Total ($F(2) = 3.009$, $p = 0.064$), and a significant interaction term for the CRT Recognition RT variable ($F(3) = 4.509$, $p = 0.019$) but not for the Motor RT, DSST, or Trails (B-A) change score variables (see Table 27). Post-hoc pairwise comparisons (with Tukey's HSD correction) for the BDI-adjusted change scores followed a similar pattern to prior analyses, with the most significant difference on the found between the Mecamylamine and Scopolamine challenges on the CRT Total change scores ($p= 0.022$), a trend-level difference between these same challenges on the CRT Recognition RT change scores ($p = 0.069$), and the BDI-adjusted SRT Total mean change scores on the Scopolamine day were significantly different from both the Mecamylamine ($p <0.001$) and Meca + Scop ($p = 0.003$) challenges.

			Main Effect (Drug)		Drug x Group	
		df	F	p	F	p
Choice Reaction Time Task	*Total RT*	3	3.903	0.015 *	2.599	0.064 †
	Recognition RT	3	3.505	0.023 *	3.943	0.023 *
	Motor RT	3	2.232	0.097 †	1.225	0.312
Digit Symbol Substitution Task	*Total Correct Symbols*	3	1.956	0.134	1.185	0.326
Trails (B-A)	*Completion Δ (B-A)*	3	2.567	0.066 †	1.313	0.282
Selective Reminding Task	*Total Words Recalled*	3	4.071	0.012 *	4.309	0.009 *

TABLE 25: Repeated Measures Mixed Model ANCOVA Table (incl. BDI as Covariate), Unadjusted Cognitive Scores (†= p <1.00, *= p <0.05, ** p < 0.001)

			Main Effect (Drug)		Drug x Group	
		df	F	p	F	p
Choice Reaction Time Task	*Total RT*	2	1.295	0.289	3.009	0.064 †
	Recognition RT	2	1.956	0.159	4.509	0.019 *
	Motor RT	2	0.356	0.704	0.582	0.565
Digit Symbol Substitution Task	*Total Correct Symbols*	2	0.723	0.494	1.217	0.310
Trails (B-A)	*Completion Δ (B-A)*	2	2.359	0.112	1.022	0.372
Selective Reminding Task	*Total Words Recalled*	2	4.202	0.025 *	3.955	0.030 *

TABLE 26: Repeated Measures Mixed Model ANOVA Table (incl. BDI as Covariate), Change Scores (†= p <1.00, *= p <0.05, ** p < 0.001)

Discussion

This research study was intended to evaluate how chronic HIV infection impacts the neuronal cholinergic receptor and whether this interaction is important for the age-related cognitive impairments of HAND, a disorder that affects nearly 60% of the HIV-positive population over the lifespan. Better understanding of the neural underpinnings of these symptoms is an important future direction for HIV-related research, especially as these individuals age. I found a significant difference in cognitive performance between the HIV-positive and HIV-negative cohorts across a number of cognitive domains during the Placebo challenge: most notably attention, psychomotor processing speed, and episodic memory, demonstrating significant performance deficits even in otherwise stable patients. In addition, examining each group's cognitive performance under the influence of the three active drug challenges, Mecamylamine, Scopolamine, and Mecamylamine + Scopolamine, reveals to what degree cholinergic blockade differentially affects particular cognitive performance domains, and suggests that changes in cholinergic systems may play a role in the symptoms of HAND.

Participant Characteristics and Vital Signs

Pre-study sample characteristics for the HIV-negative and HIV-positive cohorts of this study were consistent with prior literature on some metrics, and inconsistent on others. Participants in this study were well-matched on age, racial distribution and years of education. Chi-squared analysis for our sample showed a sex difference between the two serostatus groups, with significantly more HIV-negative women and HIV-positive men enrolled. Though the prior literature shows there are indeed known sex differences on measures of cognitive performance (Evans and Hampson 2015, McCarrey, An et al. 2016), mood (Byrne 1981, Wisniewski, Apel et al. 2005, Leach, Christensen et al. 2008), and susceptibility to HIV (Semple, Patterson et al. 1996, Kaushic, Roth et al. 2011) it is unclear whether this played a role in the cognitive data results reported here.

The final study cohort included similar numbers of APOE-e4-positive participants in both serostatus groups. In the general population, the frequency of the three allelic variants of APOE is ε-2: 8.4%, ε-3: 77.9%, and ε-4: 13.7% (Farrer, Cupples et al. 1997). The frequency of APOE allelic variants are not known specifically in the HIV-positive population. However, prior literature has identified that the presence of at least one e4 allele, is known to confer increased risk for the development of Alzheimer's Disease (AD), and also to confer higher-still risk for increased severity and faster progression of cognitive impairments and dementia in adults living with HIV (Green, Masliah et al. 2005, Chang, Andres et al. 2011, Wendelken, Jahanshad et al. 2016) as well as altering susceptibility of CD4+ cell populations to HIV infection in in vitro studies (Burt, Agan et al. 2008). Though both groups had relatively similar scores on the subjective memory measure, the MFQ, the HIV-positive group endorsed significantly higher depressive symptoms, consistent with the HIV-positive's populations higher incidence of mood symptoms and disorders (Semple, Patterson et al. 1996, Au, Cheng et al. 2008, Dal-Bo, Manoel et al. 2015).

Vital signs were collected several times over the course of each study challenge day, to monitor participant safety while under the influence of the study medications and to determine if the study medications were having the intended physiological effects. There were no baseline differences in vital signs between the groups. Mecamylamine had the expected effect on both serostatus groups of lowering blood pressure and increasing pupil size (Newhouse, Potter et al. 1994, Shytle, Penny et al. 2002). On the Scopolamine challenge day, the HIV-negative group had a larger drop in heart rate and near-significant increase in pupil size, while the HIV-negative group had very little change in vital signs between the time of administration and the initiation of the tasks, aside from an increase in pupil size. On the Meca + Scop challenge day, while all the vital signs of the HIV-negative group were significantly affected by the study medications, nearly all the vital signs in the HIV-positive group were largely unaffected.

Initial examination of the vital signs data suggested a potential group difference in the impact of the challenge medications show reduced effects on certain parameters as the HIV-positive group seemed to be less physiologically reactive to Scopolamine relative to the HIV-negative group. Scopolamine is primarily metabolized by the cytochrome p450 CYP3A (Renner, Oertel et al. 2005). However, the anti-retroviral medication class of Protease Inhibitors (PI) are known to decrease CYP3A metabolic activity (Ernest, Hall et al. 2005), as does HIV itself (Granfors, Wang et al. 2006, Kis, Sankaran-Walters et al. 2016), perhaps explaining why the reduced effects on vital signs were reduced. In contrast, mecamylamine is excreted renally un-altered, and our data shows that participants physiologically responded to this challenge medication similarly in terms of vital signs and pupillary dilation (Allanby and Trounce 1957, Alvarez-Jimenez, Baakman et al. 2017)). Protease Inhibitors are commonly added to regimens as a supplemental drug resulting in slower metabolism of anti-retroviral medications in the body and increase their efficacy (Palella, Delaney et al. 1998). However, the vital sign ANOVA results for mecamylamine and scopolamine challenge days across the seven timepoints after the administration of the study challenge drugs, would seem to contradict this possibility: there were no significant timepoint x group interaction terms found on these two challenge days, which would have indicated that physiologically, there was a difference in how the study medications were absorbed and metabolized between the participant groups. All of the HIV-positive participants in this study were taking at least one PI medication, and remained on their ART regimens throughout the study. In light of this, one might assume that the HIV-positive participants might have had a more significant physiological and cognitive reaction to scopolamine than the HIV-negative group, but that was not the case. All of the HIV-positive participants were taking some form of hypertension medication, which may have been another factor in the reduced physiological effects of the study medications

This result also suggests no meaningful difference in blood brain barrier integrity in this sample, which could have otherwise increased the access of the study drugs to the CNS. It has been previously shown (and discussed in this dissertation) that one of the pathological consequences of CNS

HIV infection is decreased integrity and increased permeability of the BBB as neurovascular epithelial cells and astrocytes are destroyed by the proliferation of the HIV virus (Power, Kong et al. 1993, Calcagno, Atzori et al. 2016, Rahimy, Li et al. 2017), though with this small sample and the study design, investigation of this factor was not possible. It is also possible that the absorption and efficacy of these medications could have been affected by participant medications other than ART drugs, as a number of the enrolled participants (particularly in the HIV-positive cohort) were on psychotropic medications for mood disorders (e.g. major depressive disorder, generalized anxiety disorder, bipolar disorder) and mild cardiovascular disorders (e.g. hypertension, hyperlipidemia) at the time of their study participation. Both of these comorbid complications have been shown to occur at a higher rate in the HIV-positive population, though it remains to be determined if these drugs played a factor in either the cognitive or physiological effects of these anti-cholinergic medications in this study

Mecamylamine Drug Challenge

I found significant effects of the nicotinic antagonist, mecamylamine, particularly on the Choice Reaction Time task, which relies on the engagement of attention to the presentation of the stimulus, as well as motor performance to successfully respond to the stimulus. On this task, the HIV-positive group performed significantly more slowly relative to the HIV-negative group on the placebo challenge day, and change score analysis showed that this difference was exaggerated after the Mecamylamine active drug challenge. and I also found evidence of a significant interaction between HIV status and drug challenge on this measure. These results support the hypothesis that there may be a relative nicotinic receptor deficit in the brains of HIV-positive individuals, resulting in greater effect of the nicotinic antagonists, consistent with the literature of the effects of mecamylamine in other, non-HIV related aging and dementia patients (Dumas, Saykin et al. 2008, Voss, Thienel et al. 2010, Baakman, Alvarez-Jimenez et al. 2017).

The CRT Task as used in this study primarily assesses and speed of orientation to an external stimulus as well as motor processing speed, to accurately and quickly execute a response to that stimulus. Serostatus group differences were seen on the placebo/inactive drug challenge day, and subsequently under the influence of mecamylamine, these differences were magnified in the HIV-positive cohort compared to the HIV-negative participants. Attention has been shown to be modulated by stimulation of nicotinic cholinergic receptors (Sarter, Bruno et al. 2003), whereby successful performance on attentional orienting or reaction time tasks like this one coincide with increases in acetylcholine release and nicotinic receptor activity (Avery, Dutt et al. 2014) and can be enhanced with nicotinic agonists (Thiel and Fink 2008) or impaired when receptors are blocked, dysfunctional, or lost (Thienel, Voss et al. 2009, Guillem, Bloem et al. 2011). As previously discussed, increased vulnerability to the negative effects of nicotinic antagonists on cognitive performance such mecamylamine may reflect a decline in nicotinic receptor system integrity, either due to older age (Newhouse, Potter et al. 1994) or to progressive neuropathological damage to these synapses (Newhouse, Potter et al. 1994) or both. In the case of these present results, it may indeed be both, as I found a significant interaction between the effects of the drug and the effects of HIV-status on this task, and this interaction remained even with the adjustment for both age and BDI score.

On the Connors CPT, analyses of the unadjusted performance scores and change scores revealed a significant difference on the Beta variable, which was higher in the HIV-positive group after all three drug conditions (though these differences were only statistically significant on the Mecamylamine challenge day). Higher beta ('response style') indicates a more cautious response pattern on the task, with participants tending to respond to the presentation of the letters more slowly to avoid errors, versus responding rapidly and less accurately (Conners, Epstein et al. 2003). This result, combined with the overall slower "Hit" reaction time in the HIV-positive group, may be partly due to age-related slowing, which is seen in the normal population (Lamb, Correa et al. 2016) and in the HIV-positive population (Branas, Jimenez et al. 2017). The administration of the drug mecamylamine may

potentially be further exacerbating this slowness in the HIV-positive cohort, affecting both cognitive processing speed and physical motor response to the task stimuli, relative to the HIV-negative group, which supports the hypothesis that increased cognitive vulnerability to anticholinergic medications may reflect increased risk of subsequent decline in cognitive functioning.

Scopolamine Drug Challenge

Under the influence of the muscarinic antagonist scopolamine there were also a number of cognitive task results on which performance differences were seen between serostatus groups when the unadjusted group performance means were examined, though when controlling for placebo condition group differences, only change scores on the SRT and CPT remained statistically significant. The anticholinergic drug scopolamine affects episodic and working memory ability (Richardson, Miller et al. 1985, Koller, Satzger et al. 2003). When comparing placebo performance to scopolamine on the SRT in this study cohort, there was a significant decrease in the number of words recalled on average in the HIV-negative group on the Scopolamine challenge day, and subsequently a significant difference in the change scores between serostatus groups under this active drug condition with a greater proportionate change in the HIV-negative group. Large differences in the short-term memory performance between the serostatus groups on the placebo day may indicate that while there was a large margin of performance impairment in the HIV-negative group, the HIV-positive group's poorer performance may be evidence of a floor-effect of both their memory and vulnerability to the anti-muscarinic medications, as the data show that they performed relatively similarly across all three drug challenge conditions: potentially, as a consequence of their HIV status they already cannot effectively store as much information, and as a consequence of their increased vulnerability to this anti-cholinergic medication, performance on this task didn't have any further to fall.

On the CPT, there was a significant group difference in the change scores for commission errors, with the HIV-positive group committing fewer errors relative to the HIV-negative group. Change

scores for Hit reaction time approached statistical different between the groups, with the HIV-positive group responding more slowly to stimuli over the course of the task. These differences (commission errors and slower reaction time) under the influence of scopolamine may reflect a trade-off between speed and accuracy.

Mecamylamine + Scopolamine Drug Challenge

Under the combination active drug condition, Mecamylamine + Scopolamine, several group differences were found when comparing unadjusted group mean performance on the cognitive battery. Prior literature suggests that the combination of both nicotinic and muscarinic antagonism should produce more significant impairments across more domains of cognition than either one drug or the other alone (Little, Johnson et al. 1998). However, on the Meca + Scop challenge day, only the Trails (B-A) change scores were statistically different between the serostatus groups. Unadjusted means were consistently higher in the HIV-positive group across all three drug challenge conditions (increased Δ between the Trails B and Trails A completion times is a measure of impaired executive functioning required for the letter-number switching on the Trails B), suggesting impaired strategy switching, an indicator of executive dysfunction. Examining the unadjusted values suggests that slower performance on this task may indicate a relative deficit in the HIV-positive individuals of the synergistic functioning of the nicotinic and muscarinic receptor systems necessary to support normal cognitive performance, consistent with the pattern of enhanced impairment when both systems are blocked vs one or the other. Though the outcome measure for this two-part task is ultimately speed of completion, the larger difference in speed between the Trails A and Trails B tasks reflects the executive functioning component of the Trails B task (Reitan 1958), requiring participants to switch between two ordered sets, an ability that is seemingly more impaired in the HIV-positive group relative to the HIV-negative group, according to our findings. Executive functioning deficits are some of the major domains affected by aging (Oosterman, Vogels et al. 2010, Hobert, Niebler et al. 2011, Roldan-Tapia, Garcia et al. 2012) and CNS-HIV infection and HAND, (Sacktor, Skolasky et al. 2010, Iudicello, Woods et al. 2012, Van

Dyk, Golub et al. 2015). Decreases in cognitive flexibility and executive functioning ability with age and HIV status have been associated with underlying changes in fronto-striatal connectivity (Morgan, Weber et al. 2012, Ipser, Brown et al. 2015) and these results would seem to suggest that cholinergic dysfunction may play a role in the presentation of these symptoms in older HIV-positive adults.

Relative Influence of Age, BDI Score, on the effects of Cholinergic Blockade

The previously discussed analyses were able to reveal which performance outcome variables differed due to HIV-status between the groups, and within each group, which performance outcome variables were affected more or less by the different challenge medications. We performed ANOVA calculations to evaluate to what degree these two factors, HIV-status (negative/positive) and drug challenge (Mecamylamine/Scopolamine/Meca + Scop) exerted a combined effect on cognitive performance (Drug x HIV interaction). Subsequent ANCOVA analyses were intended to evaluate whether two additional factors known to impair cognitive performance: age, and depression symptoms (BDI) would strengthen or weaken the significance of this interaction between Drug and HIV status on cognitive performance variables.

When examining the ANOVA results the Drug x HIV interaction term was consistently found to be significant on the CRT Total RT, Recognition RT, and SRT Total Words Recalled measures, essentially showing that not just the effects of the study medications alone, but also the specific enhanced effects of the study medications in the HIV-positive group interact, resulting in poorer performance than would be expected as a consequence of the study drug, or their HIV-status alone. Previous studies have showed that nicotinic receptor functioning is necessary for an individual to attend to novel stimuli optimally, and thus nicotinic blockade will impair this ability (Min, Moon et al. 2001, Poltavski and Petros 2006, Rusted, Sawyer et al. 2009, Thienel, Voss et al. 2009, Howe, Ji et al. 2010, Demeter and Sarter 2013). This result is consistent with the hypothesis of a deficit in nAChR system functioning in the HIV-positive participants. As previously shown, when nicotinic receptor availability

and function is decreased, individuals are more sensitive to the effects of antagonist blockade and performance is negatively affected (Newhouse, Potter et al. 1992, Newhouse, Potter et al. 1994, Newhouse, Potter et al. 1994, Levin 1996, Little, Johnson et al. 1998). It cannot be established from this study whether this result reflects dysfunction or loss of these receptors on the target cells, however it would be helpful in the future to examine density, connectivity, or binding activity at these receptors with the use of MRI or PET imaging, in conjunction with cognitive testing.

When examining the age- and BDI-adjusted ANCOVA analyses, it appears that while the age covariate increased p-values for each of the significant interaction terms, BDI score did not alter significance of the interaction terms, in the unadjusted or change score analyses. This may indicate that the cognitive impairments, and vulnerability to these medications, are not necessarily a consequence of simply having more depressive symptoms, but are instead indicative of an underlying neurobiological difference between the serostatus groups, that underlies both the deficits in their objective cognitive performance, and possibly also their subjective appraisal of those deficits as well. Prior literature has often attributed differences in subjective memory impairments to an artifact of mood symptoms, however in these analyses suggests that while HIV status and age are factors that significantly contribute to performance deficits, the effects of mood symptoms are less important.

Evidence for a Baseline Cholinergic Deficit in the HIV-Positive Brain

Overall, the most consistent results in this study were seen on the Choice Reaction Time task, which assesses attention and motor processing speed (Godefroy, Roussel et al. 2010). Performance on this task was most significantly affected by the nAChR antagonist mecamylamine, particularly in the HIV-positive cohort. The results, along with the results on the SRT and CPT support the hypothesis of a relative impairment of the nicotinic receptor system in chronic HIV-positive adults relative to HIV-negative adults, specifically affecting the cognitive domains of motor processing speed, attention, and executive function.

Previous studies have indirectly suggested that a baseline nicotinic receptor system deficit may be a mechanism that may explain the high prevalence of smoking and nicotine use in this population relative to the general population (Wojna, Robles et al. 2007, Mdodo, Frazier et al. 2015), as a form of self-medication. Additionally, with previous evidence that nicotinic cholinergic stimulation has had positive cognitive effects on outcomes in other disorders with secondary effects on cognition (such as schizophrenia, depression, ADHD, and others) (Levin 1996, Howe and Price 2001, Min, Moon et al. 2001, Engeland, Mahoney et al. 2002, Poltavski and Petros 2006, Dawkins, Powell et al. 2007, Newhouse, Kellar et al. 2012, Bain, Robieson et al. 2013), stimulating nicotinic and muscarinic cholinergic receptor systems maybe a therapeutic target to alleviate some cognitive deficits in this population as well.

One potential reason for the enhanced performance and physiological differences observed under the influence of the cholinergic antagonist medications could be that damage to the neuronal systems of the HIV-positive group that their brains could have already started rewiring to compensate for impairments in other areas. It has been suggested that the cholinergic system is a compensatory signaling systems in the human brain, increasing signaling output to maintain attention and focus in individuals experiencing normal or pathological cognitive impairments (Nordberg, Larsson et al. 1983, Dumas and Newhouse 2011). Changes to the integrity of the cholinergic system could exceed the compensatory capabilities of the system, making it more difficult (relative to the HIV-negative adults) for the HIV-positive adults to perform cognitive tasks while under the influence of the study medications impairing cholinergic neurotransmission. Though the current study design cannot definitively show this, group differences could potentially be non-specific disease-state mediated differences, a consequence of the comorbid systemic disorders that can accompany an HIV-positive diagnosis, or a result of a complex cocktail of ART treatment over the years, or some combination of these factors. It is unclear from this study design and methods whether these results reflect dysfunction or loss of these receptors

on the target cells and cellular projections to central targets, however for future inquiry it would be helpful to evaluate a similar reaction time task in a similar design with the additional use of MRI or PET imaging to determine which theory is correct.

Limitations

A small sample size, coupled with participant performance variation in the HIV-Positive cohort may have contributed to trend level differences on a number of this study's outcome measures, combined with the small overall N. Other limitations to this study included high rates of screen failure, due to a variety of reasons: in particular heavy nicotine use, severe undetermined cognitive disorder, untreated Axis 1 disorders, and inconsistent ART adherence. In addition to a relatively small study, the participants in this study were middle aged on average, in both serostatus groups. The anti-cholinergic medications in this study are known to have more significant effects on older and more impaired individuals (Zemishlany and Thorne 1991, Molchan, Martines et al. 1992, Newhouse, Potter et al. 1994, Tariot, Patel et al. 1996, Little, Johnson et al. 1998, Dumas, Saykin et al. 2008). The current study participants were mostly middle-aged (mostly in their 40's and 50's), which may be early to detect significant changes in cognitive status, within or between the study groups. It's possible that with a higher age cut-off (50+) for the study cohort, the differential effects of these medications would have been more pronounced, allowing for more clear group differences in performance, a consideration for future research design, and this may be possible in the future as the HIV-positive community continues to successfully age.

In addition to their HIV disease, several of the HIV-positive participants suffered from numerous other medical and psychiatric disorders, including but not limited to prior hepatitis, hypertension, bi-polar disorder, and more. These disorders and the relevant medications may have additional effects on their cognitive status, as well as their responses to the study medications: from changing how quickly the study drugs are metabolized, how well they are absorbed and able to access the brain parenchyma,

to interactions with the study drugs and existing standing regimens, which could reduce or enhance the effects of these medications on their cognitive functioning, altering the outcomes.

Future Directions and Considerations

This study has shown that physiological and cognitive response to anti-cholinergic drug challenge is altered between HIV-negative and HIV-positive adults, and that the cholinergic receptor system in the brains of HIV-positive adults is more sensitive to blockade than seronegative adults. The results support the hypothesis that cholinergic receptor system dysfunction may underly the HIV-related impairments of speed, attention, and executive functioning. It would be important to replicate these results in a larger study, or with a higher age range, to better understand how these results could serve as groundwork for further exploration of the role of the cholinergic system in the aging brains of HIV-positive adults. For instance, cholinergic functioning may serve as a therapeutic target to help slow or reverse the course of HAND symptoms over time, such as daily transdermal nicotine patches, a relatively non-invasive, accessible, and potentially beneficial intervention in patients experiencing the effects of both normal and pathological cognitive aging processes, as has been previously shown (Parks, Young et al. 1994, Levin, Conners et al. 1998, Min, Moon et al. 2001, McClernon, Hiott et al. 2006, Poltavski and Petros 2006, Barr, Culhane et al. 2007, Barr, Culhane et al. 2008, Gandelman, Kang et al. 2018). It is also worth noting that the Acetylcholine receptor system is not singularly responsible for the processes of cognitive aging, in the HIV-negative or HIV-positive population. Though it was not directly addressed in this study design, future inquiries into the contribution of other neurotransmitter systems, such as the dopaminergic receptor system, is additionally warranted. Evaluating dopamine receptor density or activation concurrently with cognitive performance measures, may help to determine whether there may additionally be altered interactions between the two that may reveal more information about the complex interplay between neurobiological circuits of mood, age, motivation and cognition that sufficiently explain some of the other symptoms and cognitive consequences of aging with HIV/HAND.

Conclusions

Overall, this study found that temporary cholinergic blockade had a greater relative effect on cognitive performance in the HIV-positive participants, relative to the HIV-negative participants, on measures of attention, motor processing/speed, accuracy and executive functioning. Consistent with prior literature, the domains of verbal episodic memory and fluency were relatively unaffected, once group differences in performance on the placebo/inactive challenge day were taken into account. Effects of the nicotinic receptor antagonist were somewhat more effective in producing cognitive impairments compared to the muscarinic receptor antagonist, though results under the combination drug challenge suggest that increased vulnerability of both cholinergic subsystems play a role in the cognitive deficits that are associated with decreased integrity of this receptor network. Future studies to build on these results that can combine cognitive, biomarker, and drug challenge models would be ideal to fully understand how the cholinergic system, along with other neurotransmitter circuits, systemic factors, and lifestyle factors, interact to produce the accelerated cognitive deficits associated with chronic CNS HIV-infection and the symptoms of HAND.

This is the first study to directly evaluate the effects of anti-cholinergic medications in an HIV-positive population, and provides important insights to better understand one of the neural circuitry systems associated with chronic HIV infection on the brain. I believe that this pilot study has shown important findings regarding the involvement of cholinergic mechanisms in cognitive processes in this population, and further studies incorporating other research methods such as imaging or longitudinal medications, are a necessary next step. Future MRI or PET studies that can directly assess cholinergic circuitry, receptor density/binding, or differences in basal forebrain volume, would allow researchers to more directly determine whether observed cognitive deficits correspond with biological markers of neural damage in adults living with HIV and allow future researchers to better evaluate deficits in muscarinic and nicotinic receptor system function. As the HIV-positive population continues to age,

answering more of the questions that focus not only on their primary disease markers, but also markers of mood and cognition, will hopefully lead towards not only mitigating the spread of HIV itself, but ensuring that those who now are living with this disorder can maintain the best possible quality of life for as many decades as possible.

CHAPTER IX

DISSERTATION SUMMARY AND IMPLICATIONS

In the decades since the beginning of the HIV epidemic, various lines of research have explored a wealth of topics pertaining to survival with this disease, from stopping the proliferation of the virus via ART medications, monitoring and managing side effects of both the disease, and subsequently, some of the treatments, to understanding, identifying, and controlling co-morbid risks conferred by living with a dysfunctional immune system. As methods have been revised, updated and improved, HIV in the western world can now be considered in many respects, a chronic disease, without necessarily being a death sentence, as it was in the initial emergence. Following these successes, the field has turned towards not just survival, but quality of life with HIV, which is especially important as the life expectancy of HIV-positive adults continues to steadily increase.

Reaching old age with HIV is still a relatively recent phenomenon, and as such, much of the research concerning older adults living with HIV is in the early stages or as-yet ongoing, leaving many as yet unanswered questions. Many of the individuals currently in their 50's and 60's with the disease are survivors of the initial epidemic, though there are also many new or recent infections in that age bracket as well. Whether diagnosed positive for a year, or more than 20 years, older HIV-positive adults face the inevitable combination of age-related, and HIV-related effects on their cognitive abilities, and the more information that researchers can provide, the better chance these individuals have a maintaining a healthy, independent lifestyle comparable to that of their HIV-negative counterparts.

The studies designed and executed for this dissertation sought to examine memory, attention and cognitive functioning in middle aged and older adults living with HIV, to better understand the neurobiological underpinnings of chronic CNS HIV Infection, specifically the involvement of the

acetylcholine neurotransmitter and receptor system. With Study I, the goal was to ascertain whether short, clinic-based routine cognitive assessment was 1. Feasible, and 2. Clinically meaningful to reveal early signs of cognitive dysfunction based on objective and subjective cognitive measures. We found our short battery to be quite useful to identify low-level early cognitive changes without major disruption of clinic traffic, which may be a worthwhile addition to regular HIV-medical monitoring of blood biomarkers, mood symptoms, and medication management. We also found that subjective complaints significantly correlated with objective episodic memory and stimulus recognition performance in HIV-positive patients, but not the HIV-negative patients, suggesting that though the validity of SMCs may vary in healthy study samples, they may have more validity in a patient population where even minor changes may indicate progression of underlying pathology.

Study II built on those findings, expanding the cognitive battery, and adding in the Anti-Cholinergic Drug Challenge, to determine whether these more significant cognitive impairments in the HIV-positive population could be linked to a baseline deficit in AChR system functioning, which is necessary for normal attention, memory, and executive processing. In this study, we found 1. Expected serostatus group performance differences on the Placebo/Inactive challenge day on a number of screening and cognitive outcome measures, and 2. that the anti-cholinergic challenge medications, scopolamine and mecamylamine (muscarinic and nicotinic AChR antagonists, respectively) affected the serostatus groups differently. However, there was a clear increase in the effects of the mecamylamine challenge on the HIV-positive group on attention and reaction time tasks, and significant interactions between HIV-status and mecamylamine on measures of processing speed and attention with and without age as a covariate. Attention to stimuli and speed of response to that stimuli is a necessary basic function that underlies many other domains of cognition, so evidence of an enhanced effect of this drug based on HIV-status supports the hypothesis that part of the pathology of CNS-HIV is to decrease efficiency of neurotransmission in the AChR system, which then could underlie other more

serious deficits as time progresses, widening the gap between HIV-negative and HIV-positive cognitive performance.

I believe that the results reported herein are a relevant new inquiry into the significance of cholinergic receptor tone in the aging HIV-positive brain. Future work examining imaging markers such as cholinergic basal forebrain and hippocampal volume, longitudinal cognitive assessment and follow-up, clinical trials of pro-cholinergic agents such as nicotine, novel muscarinic and nicotinic direct agonists or PAMs), are all worthwhile possible future studies to continue evaluating to what extent the cholinergic receptor system is dysfunctional in chronic HIV, and what, if anything can be done to leverage this and other neurotransmitter systems to improve cognitive outcomes in older HIV-patients.

There are numerous factors that complicate brain aging in the HIV-positive population. Early CNS infiltration of the HIV virus leads to an ongoing inflammatory and neurodegenerative process that is closely linked to the cognitive impairments of HAND, which may affect older HIV-positive adults more significantly than their younger or HIV-negative counterparts. Damage to the dopaminergic and cholinergic neurotransmitter systems in particular may contribute to the particular collection of symptoms that are seen in older HIV-positive adults. Investigating and leveraging these systems individually or synergistically may lead to treatment opportunities to improve quality of life as these individuals survive into old age, by supporting their functioning for longer before impairments are noticeable. Furthermore, understanding how dopaminergic and cholinergic systems are affected, may indicate that treatments targeted at maintaining function, or perhaps protection of these regions, may lead to better cognitive outcomes as HIV-positive individuals age.

Understanding both cognitive and physiological elements of cognitive aging with HIV may lead to more concrete approaches to both identification of symptoms, and treatments to manage those symptoms, by stimulating these underlying systems to improve cognitive outcomes as HIV-positive

individuals age. In HIV positive adults, these approaches may also need to take into account how ART needs may change when administered to an older or more cognitively impaired patient. Age-related changes in blood brain barrier integrity may alter how well-established ART regimens can penetrate the CNS, though how that may affect cognition is not yet known.

Early identification, monitoring, and management of cognitive impairments and symptoms are worthwhile additions to comprehensive HIV disease management, particularly in patients who are aging into their 60's, 70's and beyond. Regular cognitive screening, particularly as the HIV-positive patient population ages, may allow both patients and caregivers time to adjust, or to consider options to remediate symptoms, before they have progressed significantly. Treatments targeting the cholinergic or dopaminergic systems are worthwhile considerations for many reasons, one in particular being that regardless of the underlying cause of cognitive impairments, they still are effective at improving cognitive performance. Age-related impairments in HIV-positive adults may respond similarly, especially in a population already at higher risk for cognitive problems. Overall, better understanding of the nuances of aging with HIV versus normal aging, and how those neurological mechanisms are identified, monitored, and managed, will allow this vulnerable population to not only survive into old age, but thrive.

APPENDIX A

Vital Signs Means Tables

HIV-Negative - Placebo									
	Arrival	**0**	**60**	**120**	**150**	**190**	**220**	**250**	**Discharge**
Systolic BP	122.64 (12.65)	120.00 (15.398)	123.45 (13.545)	124.64 (16.213)	125.00 (19.65)	127.64 (18.364)	128.18 (22.710)	121.36 (17.910)	131.82 (20.420)
Diastolic BP	74.36 (8.33)	72.82 (12.270)	73.18 (6.824)	71.64 (8.709)	76.73 (10.403)	73.36 (10.443)	76.00 (10.761)	70.36 (9.605)	77.36 (13.048)
Heart Rate	74.27 (8.403)	67.18 (6.897)	62.55 (6.846)	60.18 (7.012)	61.91 (7.778)	62.27 (5.641)	65.55 (6.846)	66.00 (8.683)	73.36 (13.408)
Respiration	15.82 (2.601)	15.82 (3.157)	19.91 (4.134)	16.18 (2.892)	15.64 (2.656)	16.00 (5.641)	15.82 (2.442)	16.18 (2.750)	16.00 (2.966)
L Pupil (mm)	3.091 (0.944)	3.091 (0.944)	2.773 (0.817)	3.00 (0.775)	3.045 (0.650)	2.955 (0.723)	2.864 (0.778)	2.636 (0.505)	2.727 (0.647)
R Pupil (mm)	3.091 (0.944)	3.091 (0.944)	2.864 (0.897)	2.909 (0.701)	3.045 (0.650)	2.955 (0.723)	2.864 (0.778)	2.636 (0.505)	2.727 (0.647)

HIV-Negative - Mecamylamine									
	Arrival	**0**	**60**	**120**	**150**	**190**	**220**	**250**	**Discharge**
Systolic BP	152.45 (86.244)	125.27 (20.790)	119.45 (19.123)	115.82 (17.577)	111.64 (14.243)	112.82 (13.378)	116.55 (16.144)	116.55 (15.208)	119.82 (15.446)
Diastolic BP	75.18 (9.474)	72.18 (11.898)	73.18 (9.998)	69.45 (8.982)	70.27 (7.862)	68.27 (5.515)	71.91 (8.348)	67.73 (9.285)	68.64 (10.661)
Heart Rate	76.64 (7.061)	70.45 (6.846)	66.09 (7.880)	68.73 (7.431)	69.55 (9.771)	68.64 (9.437)	70.09 (11.510)	73.36 (5.853)	85.64 (11.343)
Respiration	16.55 (1.572)	17.27 (2.054)	16.55 (2.697)	17.18 (2.857)	16.18 (2.442)	17.27 (3.259)	16.00 (2.683)	16.00 (1.789)	16.55 (2.544)
L Pupil (mm)	3.682 (0.717)	3.455 (0.650)	3.409 (0.736)	3.773 (0.684)	3.682 (0.560)	3.773 (0.684)	3.955 (0.961)	3.909 (0.944)	3.909 (0.944)
R Pupil (mm)	3.682 (0.717)	3.455 (0.650)	3.409 (0.736)	3.773 (0.684)	3.682 (0.560)	3.773 (0.684)	3.955 (0.961)	3.909 (0.944)	3.909 (0.944)

HIV-Negative - Scopolamine									
	Arrival	**0**	**60**	**120**	**150**	**190**	**220**	**250**	**Discharge**
Systolic BP	124.82 (15.342)	121.45 (12.660)	121.64 (15.939)	120.64 (16.213)	120.36 (18.848)	120.09 (19.796)	122.18 (19.636)	118.45 (19.806)	132.36 (24.849)
Diastolic BP	75.00 (11.402)	73.18 (8.171)	68.36 (6.021)	71.00 (8.521)	69.45 (9.554)	71.64 (9.943)	72.82 (7.973)	67.18 (9.119)	72.55 (9.059)
Heart Rate	71.64 (9.532)	71.18 (11.001)	64.09 (8.723)	58.73 (11.671)	55.45 (9.585)	56.00 (11.018)	57.82 (11.583)	59.09 (9.502)	65.91 (15.202)
Respiration	16.55 (2.55)	16.55 (2.207)	16.36 (2.157)	16.55 (2.207)	16.18 (2.442)	16.18 (1.888)	17.09 (3.015)	16.36 (1.963)	16.55 (1.572)
L Pupil (mm)	3.136 (1.00)	3.182 (1.251)	3.727 (1.329)	4.045 (1.457)	4.364 (1.451)	4.409 (1.497)	4.182 (1.189)	4.182 (1.189)	4.182 (1.189)
R Pupil (mm)	3.136 (1.00)	3.182 (1.251)	3.727 (1.329)	4.045 (1.457)	4.364 (1.451)	4.409 (1.497)	4.182 (1.189)	4.182 (1.189)	4.182 (1.189)

HIV-Negative - Meca + Scop									
	Arrival	**0**	**60**	**120**	**150**	**190**	**220**	**250**	**Discharge**
Systolic BP	126.60 (14.393)	115.82 (10.925)	123.45 (13.545)	109.36 (13.706)	108.36 (11.544)	110.64 (14.888)	111.82 (13.370)	106.82 (15.867)	112.36 (8.914)
Diastolic BP	74.40 (9.845)	69.55 (6.729)	73.18 (6.824)	63.91 (6.300)	64.45 (7.515)	65.09 (5.941)	65.18 (5.618)	61.82 (6.332)	66.82 (55.582)
Heart Rate	74.30 (10.155)	67.27 (8.284)	62.55 (6.846)	59.64 (9.698)	61.45 (11.422)	61.91 (9.53)	61.27 (9.799)	61.91 (8.561)	73.55 (13.641)
Respiration	16.60 (1.350)	16.91 (1.375)	16.91 (4.134)	15.64 (1.502)	15.82 (1.662)	15.45 (1.572)	15.64 (1.502)	15.82 (1.888)	16.18 (2.089)
L Pupil (mm)	3.10 (0.876)	3.00 (0.633)	2.773 (0.817)	3.455 (0.789)	3.636 (0.897)	3.545 (0.789)	3.500 (0.806)	3.409 (0.801)	3.318 (0.783)
R Pupil (mm)	3.10 (0.876)	3.00 (0.633)	2.864 (0.897)	3.455 (0.789)	3.636 (0.897)	3.545 (0.789)	3.500 (0.806)	3.409 (0.801)	3.318 (0.783)

HIV-Positive - Placebo									
	Arrival	0	60	120	150	190	220	250	Discharge
Systolic BP	126.00 (17.907)	125.00 (14.938)	128.50 (14.560)	129.50 (15.166)	130 (22.334)	130.00 (21.554)	127.25 (19.739)	124.75 (16.228)	125.75 (17.401)
Diastolic BP	79.00 (10.100)	79.63 (10.253)	81.75 (9.838)	80.63 (6.989)	79.63 (7.633)	80.63 (8.141)	78.75 (8.379)	74.88 (6.578)	79.88 (9.731)
Heart Rate	70.57 (10.518)	68.75 (13.112)	62.38 (10.569)	61.25 (6.944)	60.00 (8.211)	62.00 (8.452)	63.13 (9.342)	66.13 (10.829)	74.63 (8.863)
Respiration	17.14 (1.952)	17.00 (2.138)	17.25 (2.605)	16.75 (2.816)	16.75 (2.121)	17.00 (2.619)	18.00 (2.828)	17.25 (3.196)	17.50 (2.976)
L Pupil (mm)	2.429 (0.535)	2.375 (0.518)	2.750 (0.463)	2.625 (0.518)	2.625 (0.518)	2.750 (0.707)	2.625 (0.518)	2.500 (0.535)	2.500 (0.535)
R Pupil (mm)	2.429 (0.535)	2.375 (0.518)	2.750 (0.463)	2.625 (0.518)	2.625 (0.518)	2.750 (0.707)	2.625 (0.518)	2.500 (0.535)	2.500 (0.535)

HIV-Positive - Mecamylamine									
	Arrival	0	60	120	150	190	220	250	Discharge
Systolic BP	130.11 (18.065)	125.00 (18.855)	119.33 (14.543)	117.33 (10.886)	116.56 (9.084)	119.89 (21.831)	115.11 (13.513)	114 (56) 12.032)	114.33 (19.346)
Diastolic BP	77.67 (6.285)	76.89 (7.753)	78.00 (10.296)	74.11 (8.521)	74.44 (8.202)	73.22 (8.452)	75.78 (8.614)	71.33 (9.287)	73.44 (10.596)
Heart Rate	75.44 (15.693	69.00 (13.874)	67.44 (14.783)	72.44 (11.555)	74.33 (12.124)	75.56 (11.695)	75.00 (12.309)	76.22 (11.861)	86.44 (14.371)
Respiration	17.33 (1.732)	18.00 (1.732)	16.67 (2.000)	17.33 (2.000)	17.11 (2.028)	16.67 (1.00)	17.78 (1.856)	16.67 (1.414)	17.56 (1.667)
L Pupil (mm)	2.722 (0.667)	2.722 (0.667)	2.611 (0.486)	3.056 (1.1074)	3.056 (0.950)	3.056 (0.808)	3.111 (1.024)	3.111 (1.024)	3.00 (0.829)
R Pupil (mm)	2.677 (0.707)	2.667 (0.707)	2.556 (0.527)	3.000 (1.118)	3.00 (1.00)	3.00 (0.866)	3.056 (1.174)	2.944 (0.882)	2.944 (0.882)

HIV-Positive - Scopolamine									
	Arrival	0	60	120	150	190	220	250	Discharge
Systolic BP	134.86 (20.012)	129.14 (20.940)	130.29 (21.899)	129.57 (24.144)	130.14 (25.452)	132.86 (22.579)	129.00 (20.396)	125.71 (22.104)	126.14 (13.852)
Diastolic BP	80.86 (11.261)	75.14 (6.619)	77.29 (5.499)	78.29 (8.180)	75.29 (5.589)	76.71 (7.889)	74.43 (6.188)	71.00 (6.055)	73.14 (9.940)
Heart Rate	78.00 (14.142)	64.00 (6.298)	66.29 (9.621)	60.71 (10.078)	60.00 (7.188)	58.57 (6.477)	59.14 (5.728)	61.14 (6.768)	68.14 (11.082)
Respiration	16.57 (1.902)	16.57 (1.902)	16.86 (2.268)	17.71 (2.430)	17.43 (1.902)	17.14 (2.268)	16.86 (2.545)	16.86 (2.545)	16.86 (2.545)
L Pupil (mm)	2.571 (0.787)	2.571 (0.787)	2.857 (0.690)	3.00 (0.817)	3.357 (0.748)	3.429 (0.787)	3.286 (0.951)	3.357 (0.748)	3.357 (0.748)
R Pupil (mm)	2.571 (0.787)	2.571 (0.787)	2.857 (0.690)	3.00 (0.817)	3.357 (0.748)	3.429 (0.787)	3.286 (0.951)	3.357 (0.748)	3.357 (0.748)

HIV-Positive - Meca + Scop									
	Arrival	0	60	120	150	190	220	250	Discharge
Systolic BP	132.22 (13.737)	127.89 (15.252)	129.50 (14.560)	124.22 (20.235)	121.44 (14.336)	126.89 (9.360)	119.67 (10.966)	122.33 (15.937)	123.00 (16.948)
Diastolic BP	75.44 (8.932)	74.44 (9.658)	81.75 (9.838)	75.56 (6.876)	73.00 (9.50)	75.00 (8.276)	70.78 (11.189)	68.44 (6.386)	71.22 (7.965)
Heart Rate	71.67 (8.062)	65.89 (8.418)	62.38 (10.569)	65.56 (13.030)	63.00 (10.320)	65.78 (10.745)	66.67 (11.769)	68.00 (13.667)	80.22 (13.340)
Respiration	17.33 (2.646)	16.44 (1.667)	17.25 (2.605)	16.89 (3.333)	17.11 (3.180)	16.89 (2.261)	16.89 (2.848)	16.67 (2.449)	16.89 (2.667)
L Pupil (mm)	3.056 (0.882)	2.833 (0.500)	2.750 (0.463)	3.33 (0.866)	3.333 (0.707)	3.278 (0.667)	3.22 (0.667)	3.222 (0.667)	3.222 (0.667)
R Pupil (mm)	2.944 (0.635)	2.944 (0.635)	2.750 (0.463)	3.33 (0.866)	3.333 (0.707)	3.278 (0.667)	3.22 (0.667)	3.222 (0.667)	3.222 (0.667)

APPENDIX B

Change Score Adjusted Means Tables (Repeated Measures Mixed Model ANCOVA)

Change Score Repeated Measures Mixed Model ANCOVA -- Adjusted Means (Mecamylamine)					
		Age		BDI	
		HIV-Negative	HIV-Positive	HIV-Negative	HIV-Positive
Choice Reaction Time Task	*Total RT*	26.388 (39.81)	159.248 (50.86)	17.650 (47.23)	172.650 (64.71)
	Recognition RT	4.147 (7.473)	41.483 (9.546)	- 3.457 (8.19)	53.432 (11.22)
	Motor RT	22.240 (37.09)	117.765 (47.38)	21.107 (44.25)	119.547 (60.62)
Digit Symbol Substitution Task	*Total Correct Symbols*	- 7.787 (3.34)	- 6.621 (4.28)	- 7.080 (3.97)	- 7.731 (5.44)
Trails (B-A)	*Completion Δ (B-A)*	9.748 (10.30)	20.825 (13.16)	9.747 (12.51)	20.825 (17.14)
Selective Reminding Task	*Total Words Recalled*	- 0.445 (2.76)	- 1.016 (3.53)	- 2.485 (3.15)	2.191 (4.31)

Change Score Repeated Measures Mixed Model ANCOVA -- Adjusted Means (Scopolamine)					
		Age		BDI	
		HIV-Negative	HIV-Positive	HIV-Negative	HIV-Positive
Choice Reaction Time Task	*Total RT*	75.407 (40.936)	180.432 (52.29)	47.239 (47.82)	224.696 (65.51)
	Recognition RT	28.985 (17.782)	53.023 (22.71)	11.347 (20.57)	80.741 (28.18)
	Motor RT	46.422 (30.52)	127.409 (38.99)	35.892 (36.08)	143.955 (49.43)
Digit Symbol Substitution Task	*Total Correct Symbols*	- 4.341 (3.16)	- 13.892 (4.04)	- 2.746 (3.89)	- 16.399 (5.32)
Trails (B-A)	*Completion Δ (B-A)*	17.448 (14.81)	49.295 (18.91)	8.863 (17.13)	62.787 (23.47)
Selective Reminding Task	*Total Words Recalled*	- 26.096 (2.72)	- 7.706 (3.47)	- 28.665 (3.03)	- 3.669 (4.15)

Change Score Repeated Measures Mixed Model ANCOVA -- Adjusted Means (Meca + Scop)					
		Age		BDI	
		HIV-Negative	HIV-Positive	HIV-Negative	HIV-Positive
Choice Reaction Time Task	*Total RT*	106.089 (31.27)	99.431 (39.94)	85.664 (38.78)	131.528 (53.13)
	Recognition RT	45.271 (13.22)	10.931 (16.89)	37.310 (17.48)	23.441 (23.94)
	Motor RT	60.818 (26.58)	88.501 (33.96)	48.354 (31.66)	108.087 (43.38)
Digit Symbol Substitution Task	*Total Correct Symbols*	- 4.999 (2.64)	- 4.754 (3.38)	- 4.732 (3.31)	- 4.992 (4.54)
Trails (B-A)	*Completion Δ (B-A)*	8.995 (7.87)	37.071 (10.58)	10.186 (9.88)	35.137 (13.54)
Selective Reminding Task	*Total Words Recalled*	- 7.706 (3.47)	0.039 (4.32)	- 7.426 (3.84)	- 4.616 (5.26)

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