Genetic Predictors of Cognition and Prefrontal Function in Women with HIV

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THESIS

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Dedication

To my Mother and Father, Tom and Mary Beth Sundermann who have stood behind me in all my academic and personal endeavors. Thank you for all your generosity, love and support and for teaching me the value of a good education.

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SUMMARY

Within days of infection, the HIV virus enters the central nervous system (CNS) leading to neurological, cognitive and behavioral complications. Even in the era of combination antiretroviral therapy (cART), mild neurocognitive impairment persists in approximately 45% of HIV-infected individuals particularly in the domains of executive function, learning and memory. A single nucleotide polymorphism (SNP), Val158Met, of the catechol-O-methyl transferase (*COMT*) gene, impacts prefrontal-mediated cognition (i.e. executive function) and brain response through its effect on dopamine metabolism. In both healthy and clinical populations, the Val allele of the Val158Met SNP has been associated with compromised executive function performance. In addition, neuroimaging studies report an association between the Val allele and increased prefrontal activation reflecting decreased processing efficiency during working memory tasks. Similar to the Val allele, HIV infection is associated with decreased dopaminergic activity and inefficient prefrontal activation during working memory tasks. Therefore, this study involved both behavioral and neuroimaging substudies in order to determine the independent and interactive effects of HIV serostatus and the *COMT* Val158Met polymorphism on working memory performance in women and the neural systems underlying this effect. Participants were recruited from the Chicago Women's Interagency HIV Study (WIHS) Consortium. For the behavioral component, participants included 54 HIV-infected women (33 Met allele carriers and 21 Val/Val) and 33 HIV-uninfected women (12 Met allele carriers & 21 Val/Val). The sample was 81% African American and between 25 and 71 years of age (M = 40.8, SD =10.2). During their biannual WIHS visit, participants completed the 0-, 1- and 2-back conditions of the N-back, a test of working memory. We conducted a mixed-factor analysis of variance (ANOVA) to examine the independent and interactive effects of HIV serostatus and *COMT* Val158Met genotype on working memory (N-back) performance after adjusting for relevant covariates. We hypothesized that the *COMT*

Val/Val genotype and HIV-infection will be associated with poorer working memory performance in the behavioral substudy and increased prefrontal cortex activity in the neuroimaging substudy. Specifically, HIV positive women with the Val/Val genotype will show the worst performance and greatest prefrontal activation overall. In support of hypotheses, the HIV-infected women demonstrated significantly worse N-back performance compared to HIV-uninfected women ($p < 0.05$). In addition, a significant serostatus by genotype interaction ($p < 0.01$) revealed that, among Val/Val, but not Met allele carriers, HIV-infected women performed significantly worse than HIV-uninfected controls across combined N-back conditions ($p < 0.01$). Results suggest that the negative impact of HIV on working memory is driven by individuals with suboptimal, dopamine levels in the prefrontal cortex (Val/Val). For the neuroimaging component, 33 women (20 HIV-infected & 13 HIV-uninfected women) underwent fMRI assessments at the University of Illinois at Chicago Magnetic Resonance Center while completing the 0-, 1- and 2-back conditions of the N-back task. A region of interest analysis was conducted in order to specifically examine differences in brain activation between serostatus and genotype groups in brain areas reported as significantly active in previous neuroimaging studies. These areas include the prefrontal and posterior parietal cortex, anterior cingulate, caudate and putamen. Results from the imaging analysis were consistent with the behavioral finding in that serostatus and genotype significantly interacted to impact brain activation patterns ($p < 0.01$). In support of hypotheses, HIV-infected, Val/Val carriers showed significantly greater brain activation in the anterior cingulate and prefrontal regions compared to HIV-uninfected, Val/Val carriers. Discordant with hypothesis, HIV-uninfected, Met allele carriers demonstrated significantly greater brain activation in an anterior cingulate and a prefrontal region compared to HIV-infected, Met allele carriers. Findings suggest that the neural

basis underlying the working memory deficit in HIV-infected, Val/Val carriers is inefficient brain processing and the compensatory recruitment of additional neural resources. Given the Val/Val genotype is associated with decreased dopamine levels in the prefrontal cortex; it is proposed that the association between HIV infection and inefficient brain activation during working memory is driven by individuals with suboptimal, dopamine levels in the prefrontal cortex (Val/Val). Perhaps, the combination of the Val/Val genotype and HIV elicits a decrease in processing efficiency that leads to the increased recruitment of neural resources in the working memory network as task complexity increases. Overall, findings highlight dopamine dysfunction as a neural mechanism underlying HIV-neurocognitive disorders and suggest that the decrease in dopamine signaling that results from the combination of HIV and the Val/Val genotype contributes to a vulnerability to working memory impairment.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) enters the central nervous system (CNS) within days of initial infection leading to neurological, cognitive and behavioral complications. Neurocognitive deficits are a common feature of HIV/AIDS, and the frequency and severity of these deficits increase as the disease progresses (Levin *et al*., 1990). The cognitive deficits associated with HIV infection, HIV-associated neurocognitive disorders (HAND), range from subtle cognitive deficits to a marked dementia syndrome, HIV-associated dementia (HAD) (Heaton *et al*., 2011). HAND is categorized into three conditions depending on the number of cognitive domains impaired and the level of interference in everyday life. These three conditions include asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). A diagnosis of HAND requires impairment in at least two cognitive domains as indicated by performance of at least 1.0 standard deviation below the mean for age-education-appropriate norms on standardized neuropsychological tests (Antinori *et al*., 2007). A relevant and informative question is why some HIV-infected individuals develop HAND while others do not. Attention/working memory is one of the cognitive domains examined in order to determine HAND status. We will focus specifically on working memory deficits in order to investigate a genetic factor that may contribute to the vulnerability to HAND.

A. *HIV-related cognitive deficits persist in the cART era*

Combined anti-retroviral therapy (cART), the combination of two or more antiretroviral drugs, has become the standard treatment for HIV infected individuals and has led to a dramatic improvement in their overall health and longevity (Valcour & Paul, 2006). The increased lifespan of individuals with HIV highlights the importance of understanding the effects of HIV on the brain since the aging brain is more susceptible to HIV-associated cognitive impairments (Valcour *et al*., 2004). HIV and age-

associated neurodegenerative disorders may interact either additively or synergistically (Valcour & Paul, 2006).

After the advent of cART, there was a decrease in the incidence of HAD; however, the incidence and prevalence of milder forms of HAND have remained stable and may have even increased among asymptomatic HIV patients (Grant, 2008; Heaton *et al*., 2011; Woods *et al*., 2009). It is estimated that 45% of HIV-infected individuals have milder forms of HAND in the post-cART era, including ANI and MND (Heaton *et al.*, 2011). Furthermore, an observed trend in the post-cART era is the diagnosis of a HIV-related CNS disease (Dore *et al*., 1999; Sacktor *et al*., 2001) at a considerably higher median CD4 cell count compared to the pre-cART era suggesting that cART produces less protection against cognitive impairment than other AIDS-defining illnesses. In addition to changes in incidence, the characterization of HAND also changed in the post-cART era whereby, the cognitive domains most impaired by HIV shifted from motor skills and cognitive speed to memory and executive function (Heaton *et al*., 2011). HIV-related cognitive deficits are believed to persist in the post-cART era for various reasons including poor penetration of the CNS, drug resistance, poor medication adherence (Cysique & Brew, 2009) and certain brain cells serving as viral reservoirs (Kramer-Hämmerle *et al*., 2005). The HIV virus penetrates the central nervous system in the early stages of the disease (Clements *et al*., 2002). Once this occurs, the virus cannot be eradicated from infected brain glial cells due to the long lifespan and low turnover of infected brain glial cells (Nath & Sacktor, 2006). Once prescribed, many antiretroviral medications are unable to effectively enter the CNS due to poor penetration of the blood brain barrier and the subtherapeutic levels of drugs that do enter can cause the development of resistant viruses (Ellis *et al*., 2000; Price & Deeks, 2004).

Taken together, these data suggest that cognitive deficits continue to be a significant problem in HIV even after the advent of cART and can be observed in less severe disease stages (e.g., higher CD4

cell counts). With the increase in milder forms of cognitive impairment and the decrease in disease severity in the cART era, neuropsychological test performance is ascribed more validity in predicting HIV disease progression. This assertion is supported by the finding that mild neurocognitive dysfunction is a strong risk factor for HIV disease progression, poor medication adherence and encephalitis (Heaton *et al*., 2004; Cherner *et al*., 2002; Hinkin *et al*., 2004). This underscores the importance of identifying factors that increase vulnerability to HIV-related cognitive deficits.

B. *The importance of studying working memory in women with HIV*

HIV/AIDS was once a disease considered to be confined within the homosexual male population, but now the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that the global proportion of women versus men who are infected has been approximately 50% since the late 1990s (UNAIDS, 2008). Statistics from the Center for Disease Control (2008) indicate that the proportion of AIDS cases among women has nearly doubled in the past decade. The highest incidence of HIV in the United States among women is found in African Americans where the rate is nearly 15 times higher compared to White women and about four times higher compared to Hispanic/Latina women. Therefore, minority females are a particularly vulnerable population. In addition, women with HIV, especially African American women, may be at increased risk for cognitive decline due to the high prevalence of psychosocial and mental health problems, and lower cognitive reserve associated with lower education (Basso & Bornstein, 2000a; Bornstein *et al*., 1993; Maki and Martin, 2009). Despite these findings, few studies have examined cognitive function in women with HIV/AIDS (see Maki and Martin, 2009 for a review; also Richardson *et al*., 2002, 2005).

In order to diagnose HAND, the cognitive domains that need to be assessed are verbal/language, attention/working memory, abstraction/executive, memory (learning and recall), speed of information processing, sensory-perceptual, and motor skills. The current study will focus specifically on the

domain of attention/working memory. The importance of this focus emanates from findings reported by Heaton *et al.* (2004) that, among HIV-infected individuals, attention/working memory is one of the cognitive domains that most strongly and consistently predicted performance on a standardized evaluation of everyday functioning (e.g. shopping, cooking, financial and medication management). Furthermore, impairment on both the cognitive and functional evaluations was significantly associated with subjective reports of cognitive difficulties, unemployment and greater dependence in everyday activities (Heaton *et al*., 2004) suggesting that the HIV-associated cognitive deficits are clinically relevant.

The importance of studying cognition in women with HIV extends to the specific domain of working memory due to evidence of a relationship between sex steroid hormones and working memory. The prefrontal cortex is one of the highest estrogen binding sites in the human brain, with estradiol concentrations in the prefrontal cortex being approximately two times higher compared to the temporal lobe and seven times higher compared to the hippocampus (Bixo *et al*, 1995). An observational study of 73 younger postmenopausal women (mean age = 56) found evidence that hormone therapy (HT) enhanced performance on tests of working memory, and this was due to an improvement in the prefrontal-mediated processes of information updating and manipulation and not passive short-term memory (Duff and Hampson, 2000). The N-back test is a widely used and well-validated test of verbal working memory and, although some studies have found no effect of sex on N-back performance at the behavioral or neural level (Hyde *et al*, 2005; Schmidt *et al*., 2009), beneficial effects of estrogen have been reported on N-back performance. Keenan and colleagues (2001) compared performance on a cognitive test battery between nine menopausal women on HRT and 10 menopausal women without prior exposure to HT. Working memory was one of the domains where a significant difference was observed between groups whereby, the non-HRT group showed worse N-back performance compared to

the HRT group. In a pharmacological model of menopause, estrogen suppression induced by leuprolide acetate was associated with a decrease in performance on two measures of working memory, the N-back and the Letter-Number Sequencing Task (Grigorova *et al*, 2006). A neuroimaging study examined whether menstrual cycle phase modulates brain activation during the N-back task by comparing activation patterns between women in the late follicular phase, characterized by high estradiol levels and women in the early follicular phase, characterized by low estradiol levels (Joseph *et al*., 2012). Although the active brain regions did not differ between groups, better N-back performance in the late follicular phase was associated with less activation in the left hemisphere and more activation in the right hemisphere compared to the early follicular phase (Joseph *et al*., 2012). This finding suggests the recruitment of the neural networks supporting working memory may be modulated by the impact of estrogen on hemispheric asymmetry. Collectively, these studies suggest that sex can impact working memory performance by means of sex steroid hormones.

The COMT gene influences executive function through its role in dopamine function

Over half of the variance in adult cognition is accounted for by genetic markers (Plomin *et al*., 2001). In numerous reports, the catechol-*o*-methyltransferase gene (*COMT*) gene has been associated with working memory, an executive function that is responsible for the temporary maintenance and manipulation of information. This gene produces a COMT enzyme expressed throughout the brain with the highest rate of activity in the prefrontal cortex (PFC) (Garris *et al*., 1993). The enzyme is responsible for metabolizing catecholamines including dopamine, norepinephrine and catecholestrogens. A functional single nucleotide polymorphism at codon 158of the *COMT* gene coding region causes a valine (Val) to methionine (Met) amino acid substitution (Bertocci *et al*., 1991; Lundstrom *et al*., 1991). This polymorphism is commonly referred to as Val158Met (dbSNP accession rs4680) and influences activity and thermal stability of the catechol-*o*-methyltransferase enzyme (Lachman *et al*., 1996). The

Met/Met homozygotes have one quarter of the *COMT* enzyme activity as Val/Val homozygotes, with Val/Met heterozygotes exhibiting an intermediate phenotype (Lachman *et al*., 1996). As the number of Met allele increases, there is a greater reduction in *COMT* activity resulting in slower dopamine breakdown and, in turn, increased dopamine signaling. Electrophysiological studies in primates (Sawaguchi & Goldman-Rakic, 1991) and neuroimaging studies in humans (Daniel *et al*., 1991) have demonstrated that dopaminergic function has a critical role in the modulation of PFC activity, and it is through this role that dopamine heavily influences executive function abilities. More specifically, dopamine neurotransmission has been subdivided into tonic and phasic activity, where tonic dopamine activity represents the low-level, background dopamine activity that is mediated by baseline dopamine neuron firing and phasic dopamine activity represents high-level, transient dopamine release mediated by bursts in the firing of dopamine neurons (Bilder *et al*., 2004). It is believed that the *COMT* Met allele results in increased tonic dopamine and reduced phasic dopamine in subcortical regions and increased dopamine transmission cortically. This pattern of effects is hypothesized to increase stability in the neural networks that subserve working memory and executive function (Bilder *et al*., 2004).

Another proposed theory regarding the molecular basis of the effects of *COMT* genotype on executive function involves the differential effects of the D1 and D2 receptor binding. It is theorized that D1 receptor binding is analogous to phasic dopamine activity in that it is an indicator of new and important stimuli in the environment that needs to be incorporated into working memory (Vijayraghavan *et al*., 2007). D1 receptor activation results in an increased signal to noise ratio by way of diminishing neural excitation related to insignificant stimuli (“noise”) and increasing neural excitation related to significant stimuli (“signal”) (Durstewitz & Seamens, 2008). Conversely, D2 receptor binding leads to a decrease in the filtering of incoming stimuli into the PFC resulting in an increase in distracting inputs and, in turn, a decreased signal to noise ratio (Weinberger *et al*. 2001). Therefore, when a shift occurs in

the D1/D2 receptor binding ratio, PFC-mediated function can be impacted in either direction, increased or decreased signal to noise ratio. In terms of the inverted U-shaped curve theory, when PFC dopamine levels are very high or low, effects from D2 receptor binding dominate, whereas, when PFC dopamine levels are moderate, effects from D1 receptor binding dominate (Durstwitz & Seamans, 2008). The decrease in PFC dopamine levels associated with the Val allele leads to reduced D1 receptor binding resulting in a diminshed ability to maintain stimuli in working memory by way of decreased stability of the PFC. (Bilder *et al*. 2004; Winterer & Weinberger, 2004). Conversely, the increase in PFC dopamine levels associated with the Met allele leads to greater D1 receptor binding resulting in improved working memory by way of increased stability of the PFC (Bilder *et al*. 2004; Winterer & Weinberger 2004).

C. *Behavioral studies of Val158Met and executive function*

The influence of the Val158Met polymorphism on dopamine function has led to numerous studies investigating the influence of the polymorphism in disorders related to dopamine dysfunction (i.e. schizophrenia and Parkinson's disease) as well as in healthy adults. A number of studies examined the relationship between *COMT* genotype and cognitive function in a combined sample of healthy controls and individuals with schizophrenia or schizotypal personality disorder in order to compare effects between patients and controls (Egan *et al*., 2001; Goldberg *et al*., 2003; Minzenberg *et al*., 2006). Regardless of clinical status, the Val/Val genotype related to poorer performance on PFC-mediated tasks including the Wisconsin Card Sorting Task (WCST) (Egan *et al*., 2001; Minzenberg *et al*., 2006), the Paced Auditory Serial Attention Test (PASAT) (Minzenberg *et al*., 2006), and the N-back test (Goldberg *et al*., 2003). Furthermore, the detrimental effect of the Val/Val genotype on prefrontal-mediated cognition was of similar magnitude in the patients and controls; however, patients demonstrated poorer executive function compared to controls regardless of genotype (Egan *et al*., 2001;

Minzenberg *et al*., 2006). Notably, Minzenberg and colleagues reported that the variation in *COMT* genotype showed a greater effect on cognitive performance than clinical status (2006). The lack of an association between Val158Met genotype and cognitive domains other than executive function demonstrates the specificity of the effect of the Val158Met genotype on PFC-mediated cognitive function.

The relationship between dopamine levels and PFC-mediated cognition is not linear. It is proposed that the relationship between PFC dopamine levels and executive function is characterized by an inverted U-shaped curve where PFC dopamine levels that are at the peak of the curve are optimal for executive function. Conversely, dopamine levels that fall to the left or right of the peak lead to poorer performance on these tasks (Cai and Arnsten, 1997; Egan et al., 2001; Granon et al., 2000; Zahrt et al., 1997). Therefore, the increase in dopamine signaling associated with the Met allele may be beneficial or detrimental to working memory performance depending on one's basal dopamine level. For example, the dopamine dysfunction associated with schizophrenia is characterized by relative decreases in prefrontal dopamine activity (Weinberger, 1987). The reported detrimental effect of the Val/Val genotype on executive function in schizophrenia suggests that the Val/Val genotype results in an additional decrease in prefrontal dopamine levels so that levels fall further left of the curve. Prefrontal dopamine levels associated with the Met allele fall closer to the peak of the curve in schizophrenia (see Figure 1). Alternatively, the Met allele is associated with poorer working memory performance when amphetamine, a dopamine agonist, is administered to healthy adults. It is purported that the combination of the Met allele and amphetamine use pushes PFC dopamine levels to supraoptimal levels where working memory performance begins to decline (Mattay *et al*., 2003).

D. *Neuroimaging studies of Val158Met and prefrontal function*

Several neuroimaging studies examined executive function in relation to the Val158Met polymorphism in healthy and patient populations. The primary findings of fMRI studies were associations between the Val allele and increased prefrontal activation during working memory tasks in individuals with schizophrenia (Egan *et al*., 2001; Ho *et al*., 2005) and healthy adults (Bertolino *et al*., 2006; Callicott *et al*., 2000; Heinz & Smolka, 2006; Jacobs & D'Esposito, 2011; Mattay *et al*., 2003; Meyer-Lindenberg *et al*., 2006). Other brain areas that showed increased activation in relation to the Val allele include the anterior cingulate (Bertolino *et al*., 2006; Meyer-Lindenberg *et al*., 2006), the posterior parietal cortex (Bertolino *et al*., 2006; Egan *et al*., 2001; Meyer-Lindenberg *et al*., 2006), the caudate and putamen (Bertolino *et al*., 2006). The increased prefrontal activation in Val allele carriers was interpreted to represent decreased processing efficiency. In other words, the suboptimal prefrontal dopamine levels associated with the Val allele may require greater recruitment of neural resources in order to achieve behavioral performance equivalent to those without a Val allele. Imaging studies that examined the effect of the Val158Met SNP in healthy adults and schizophrenia using three genotype groups (Val/Val, Val/Met and Met/Met) found that the effects were dose-dependent such that Val/Val homozygotes showed the greatest prefrontal activation while Met/Met homozygotes showed the least (Egan *et al*., 2001; Heinz and Smolka, 2005; Meyer-Lindenberg *et al*., 2006). Collectively, these imaging studies provide strong evidence for a relationship between suboptimal prefrontal dopamine levels and decreased cortical efficiency. On a cellular level, the hypothesis is that decreases in dopamine function associated with the Val allele lead to reduced signal to noise ratios in neuronal firing (Servan-Schreiber *et al*., 1990).

Goldberg *et al.* (2003) provided insight into the specific working memory subprocesses that demonstrate sensitivity to *COMT* genotype. *COMT* genotype had no effect on the 0-back condition of the N-back test, which draws primarily on attentional processes; however, *COMT* genotype showed a

similar effect on the 1- and 2-back conditions. Both the 1- and 2-back involve full engagement of working memory processes including updating and temporal indexing of incoming stimuli; however, the 2-back involves a longer delay between stimulus presentation and recall and a greater information load to be held in working memory compared to the 1-back. The non-differential effect of the *COMT* genotype on the 1- and 2-back conditions suggests that the susceptibility of working memory to *COMT* genotype effects is related to the subprocesses of updating and temporal indexing but not to load and delay (Goldberg *et al*., 2003). Neurophysiological studies suggest that dopamine may be particularly important in these specific cognitive control processes (Crofts *et al*., 2001).

E. *HIV infection is associated with dopamine dysfunction*

HIV neuropathology is widespread in both cortical and subcortical brain areas; however, the virus has a particular affinity for the hippocampus and the frontal-striatal circuits including the basal ganglia (Aylward *et al.*, 1993; Castelo *et al.*, 2006; Masliah *et al*., 1992). HIV enters the CNS as infected monocytes and macrophages (Gendelman *et al*., 1989) where it infects microglia, additional macrophages and astrocytes (Shapshak *et al*., 1992). The infection of these cells leads to a cascade of neurotoxic events that indirectly lead to damaged neurons by way of secreted viral neurotoxins, neuroinflammation, myelin degradation and the breakdown of the blood brain barrier (Genis *et al*., 1992; Giulian *et al*., 1990, 1993; Pulliam *et al*., 1991). The neuropathology damages a variety of neural circuits; however, the dopaminergic neurons of the basal ganglia appear to be impacted in the early stages of infection (Woods *et al*., 2009). This view is in concordance with findings of a loss of dopamine cell bodies in the basal ganglia of patients with HAD (Itoe *et al*, 2000; Reyes *et al*, 1991) and decreased dopamine levels in the cerebral spinal fluid of HIV patients with and without cognitive deficits (Berger *et al*., 1994). Depending on brain region, a two to 53 percent decrease in dopamine concentrations were found in the brains of HIV-infected, HAART-treated individuals (Kumar *et al*.,

2011). Collectively, the findings suggest that HIV-related damage to dopaminergic neurons results in a decrease in dopamine function and release. Furthermore, a recent positron emission tomography (PET) study found an inverse relationship between viral burden and dopamine D2 receptors and transporters in HIV patients with cognitive impairment suggesting that dopamine function decreases as disease severity increases (Wang *et al*., 2004).

F. *HIV infection is associated with working memory deficits*

Evidence suggests that the HIV-associated dopamine dysfunction is one of the neural mechanisms underlying HAND. The basal ganglia provide dopamine innervation to the frontal lobe. Given the reliance of working memory processes on dopamine signaling in the PFC, the decrease in dopamine function in HIV infection is consistent with evidence of working memory deficits in the disease (Bartok *et al*., 1997; Hinkin *et al*., 2002; Martin *et al*., 2001; Sun *et al*., 2010; Woods *et al*., 2009). HIV-seropositive adults performed significantly worse than controls on both a verbal and spatial N-back working memory tests (Hinkin *et al*., 2002) and the WAIS-R III Digit Span (Sun *et al*., 2010). Other studies have examined working memory in drug users with HIV and without HIV and found a relationship between poorer working memory performance and HIV-infection (Bartok *et al*., 1997; Martin *et al*., 2001). More direct evidence for the dopamine dysfunction and working memory relationship was found in a postmortem study by Kumar and colleagues (2011) that reported a significant correlation between brain levels of homovanillic acid, a dopamine metabolite, and antemortem working memory performance (Kumar *et al*., 2011). Therefore, there is evidence to suggest that treating dopamine dysfunction in HIV patients with cognitive deficits may provide an avenue for effective treatment development.

Neuroimaging studies have used methods such as positron emission tomography (PET), diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS) to characterize brain

abnormalities in cerebral glucose metabolism (Rottenberg *et al*., 1996), axonal integrity (Pfefferbaum *et al*., 2009) and cerebral metabolites (Barker *et al*., 1995; Chang *et al*., 1999a; Lopez-Villegas *et al*., 1997) in HIV seropositive individuals, respectively. Very few studies have used fMRI to examine the neural correlates of deficits in working memory in HIV, especially in women with HIV. In a neuroimaging study by Chang and colleagues (2001), HIV seropositive men with early stage HAD demonstrated greater brain activation in the prefrontal and parietal regions compared to uninfected controls during the N-back test. Similar to the adverse effects of the Val/Val genotype on prefrontal processing efficiency, this increase in activation observed in the HIV-infected men is believed to represent decreased processing efficiency as a consequence of HIV-related dopaminergic dysfunction. More relevant to the current study, Ernst and colleagues (2002) examined brain activation during the N-back test in men with HIV and without cognitive impairment. Similar to the findings of Chang *et al*. (2001), increased brain activation was found in the lateral prefrontal regions in men with HIV compared to age and education-matched, seronegative, male controls. This brain activation difference was observed despite no differences in behavioral N-back performance between the serostatus groups (Ernst *et al*., 2002). Ernst and colleagues (2002) concluded that HIV neuropathology may lead to increased reliance on brain reserve to maintain normal cognitive function, and this pattern may precede clinical signs or cognitive deficits.

G. *Rationale for examining the effect of the Val158Met SNP in women with HIV*

To date, two studies have examined the effect of the Val158Met SNP on working memory function in an either all male or a mixed sex sample of individuals with HIV. Levine and colleagues (2012) found no effect of the Val158Met SNP on five neurocognitive domains (working memory, processing speed, learning, memory, motor) in a sample of 184, primarily Caucasian, men and women with HIV. Bousman and colleagues (2010) examined how use of methamphetamine, a dopamine

agonist, modulates the effect of the Val158Met SNP on neurocognitive function in 229 HIV-infected and uninfected men. Among methamphetamine non-users in both serostatus groups, there was an association between the Met/Met genotype and better executive function. Conversely, there was no positive effect of the Met/Met genotype on executive function among HIV-infected or uninfected methamphetamine users. In support of the inverted U-shaped curve, it was suggested that the combination of the Met/Met genotype and methamphetamine use leads to excessive levels of dopamine in the PFC that are not optimal for executive function performance (Bousman *et al*., 2010). Bousman *et al*. (2010) and Levine *et al*. (2011) and examined the independent effects of *COMT* genotype on cognition in HIV; however, no studies have yet examined the interactive effects of *COMT* genotype and HIV serostatus.

The effect of *COMT* genotype on working memory performance has yet to be examined among a large sample of women with HIV. The previous studies examining the effect of *COMT* on working memory in a HIV population have used either an all-male (Bousman *et al*., 2010) or a mixed sex sample comprised of 13% females (Levine *et al*., 2011). A review of *COMT* and cognitive function studies noted that study samples with a higher percentage of women show a stronger effect size of the *COMT* genotype (Tunbridge *et al*., 2006). Furthermore, animal studies show that estradiol augments dopamine activity by attenuating the amount of dopamine depletion that occurs from neurotoxins that target the striatal dopaminergic system (Becker, 2000; Dluzen, 2000; Thompson and Moss, 1994; Xiao and Becker, 1994). The proposed estrogen and dopamine relationship is supported by findings from Jacobs and D'Esposito (2011) showing that the effect of endogenous estradiol on working memory in healthy, young women is dependent on the *COMT* Val158Met genotype. This study used a cross-over design to examine working memory performance and prefrontal function in Val/Val and Met/Met genotype carriers at two different points in their menstrual cycle: menses, characterized by low estradiol levels,

and ovulation, characterized by high estradiol levels. Consistent with the inverted U-shaped curve hypothesis, Val/Val carriers exhibited better working memory performance when estradiol levels were high versus low; whereas, Met/Met carriers exhibited the opposite pattern with better working memory performance occurring during states of low estradiol levels (Jacobs & D'Esposito, 2011). Therefore, it is possible that the effect of the *COMT* genotype on working memory is, at least, partially dependent upon sex and the presence of sex steroid hormones.

H. *Significance*

The importance of studying *COMT* in HIV stems from the fact that working memory deficits are evident in HIV positive individuals, even in those receiving HAART. Studies in healthy controls and persons with schizophrenia illustrate the large impact of the *COMT* genotype on working memory performance and the importance of identifying genetic factors that further compromise working memory deficits in clinical populations. A meta-analysis reported that the effect size associated with a Val/Val versus Met/Met genotype effect on N-back performance is significantly higher in clinical (d = 0.40) versus control (d = 0.27) populations (Barnett *et al*., 2008). The motivation for examining this particular genotype in this disease comes from the large overlap in the specific cognitive and neural mechanisms shown to be affected by *COMT* in healthy adults and to be impaired in HIV. The hypothesis is that this polymorphism compounds the HIV-associated vulnerability to deficits in working memory. Treatment strategies targeting the *COMT* enzyme and dopamine transmission, including *COMT* inhibitors are under evaluation (Apud *et al*, 2007; Farrell *et al*., 2012; Giakoumaki *et al*., 2008), suggesting that future treatments may be available for HIV positive individuals and utilized based on Val158Met genotype.

The importance of studies examining gene and brain activation relationships is illustrated in the intermediate phenotype concept put forth by Meyer-Lindenberg and Weinberger (2006). The

underlying assumption of the theory is that the more behavioral a phenotype, the less directly it can be predicted by a genotype due to the multitude of gene-gene and gene-environment interactions contributing to a phenotype. Therefore, Meyer-Lindenberg and Weinberger (2006) describe the strategy of examining genotypes in relation to physiological, quantitative traits that more directly reflect biological processes such as brain activation. It is likely that the effects of genetic variation, such as variations within *COMT*, are more directly observable when comparing brain function rather than focusing solely on working memory performance. Therefore, associations between genotype and brain function may be most evident in imaging studies, especially for carriers of a single risk allele even if those carriers show no behavioral effects. By linking genetics to brain function in imaging studies, biological processes are identified that make a unique contribution to overall behavior and clinical outcomes (Meyer-Lindenberg and Weinberger, 2006). The identification of these biological processes is the initial step in the development of targeted treatments and clinical care.

This cross-sectional study involves both a behavioral and an imaging substudy in order to examine the effects of HIV and the Val158Met allele on working memory test performance and the neural systems underlying performance. The N-back test was used to assess working memory because it has shown sensitivity to abnormalities in PFC function in individuals with HIV (Chang *et al*., 2001; Ernst *et al*., 2002) and has reliably shown sensitivity to differences in PFC activation patterns across *COMT* genotype groups in healthy adults (Bertolino *et al*., 2006; Heinz and Smolka, 2006; Ho *et al*., 2005; Minzenberg *et al*., 2006) and schizophrenia (Egan *et al*., 2001; Minzenberg *et al*., 2006). Given that both HIV and schizophrenia are characterized by hypodopaminergic function in the PFC, it is reasonable to hypothesize that the impact of *COMT* genotype on working memory performance will be similar in both disease states. We predict that the Val/Val genotype will compound the negative effects

of HIV infection so that HIV-infected women with the Val/Val genotype will show the worst performance on the N-back and in the neural systems underlying working memory performance.

G. *Statement of Aims and Hypotheses*

Specific Aim 1: to examine the behavioral effects of HIV and the *COMT* Val158Met polymorphism on working memory performance in midlife women.

Hypothesis 1a: Women with HIV will perform more poorly than women without HIV on the N-back test.

Hypothesis 1b: Women with the Val/Val genotype will perform more poorly than Met allele carriers on the N-back test.

Hypothesis 1c: The relationship between the Val/Val gene and poorer working memory performance will not differ between HIV-infected and uninfected women; however, the Val/Val genotype will compound the negative effects of HIV so that HIV-infected women with the Val/Val genotype will show the worst performance

Specific Aim 2: to use fMRI to determine the effects of HIV serostatus and *COMT* Val158Met genotype on patterns of neural activation during the N-back test.

Hypothesis 2a: Women with HIV will show greater brain activation than women without HIV on the N-back test.

Hypothesis 2b: Women with the Val/Val genotype will show greater brain activation than Met allele carriers on the N-back test.

Hypothesis 2c: The relationship between the Val/Val genotype and greater activation will not differ between HIV-infected and uninfected women; however, the Val/Val genotype will compound the effects of HIV on activation so that HIV-infected women with the Val/Val genotype will show the greatest activation overall.

II. METHODS

A. *Participants*

1. *Behavioral & Imaging Studies*

Participants were recruited from the Women's Interagency HIV Study (WIHS), the nation's largest cohort study of the natural and treated course of HIV in women. WIHS was established in 1993 and has enrolled over 4,000 HIV-infected and uninfected women to date. Participation in WIHS includes comprehensive evaluation of physical and mental health every six months (Barkan *et al*., 1998). Although there are six clinical WIHS centers throughout the United States, only women participating in the Chicago WIHS Consortium were invited to participate. Inclusion criteria for all participants were: (1) the ability to speak and read English; (2) 18 to 73 years of age; (3) the ability to give informed consent; and (4) previously consented to genetic testing in WIHS. Exclusionary criteria for all participants were: (1) self-reported history of dementia or other AIDS-defining disorders affecting the CNS (e.g. toxoplasmosis, cryotococcal meningitis, CNS lymphoma, encephalitis); (2) uncontrolled diabetes as evidenced by persistent fasting blood glucose levels greater than 126 mg/dl at more than one visit within the past two years and no initiation of diabetic medication or a change was made in medication within the past 6 months; (3) history of any other self-reported endocrine or systemic disease that affects cognition (Parkinson's disease, multiple sclerosis cerebral palsy, syphilis) (4) closed head injury with loss of consciousness for greater than one hour; (5) open head injury of any kind; (6) seizure disorder; (7) currently pregnant; (8) currently taking antipsychotic medication; (9) fewer than eight years of formal education; (10) evidence of acute drug or alcohol intoxication or withdrawal at testing; (11) use of a stimulant drug in the past six months; (12) history of schizophrenia; (13) bipolar disorder; (13) any problems with maintaining attention over long periods of time; (14) active interferon treatment for

the Hepatitis C virus; (15) history of stroke or cerebrovascular disease or (16) vision problems that cannot be corrected with eyeglasses or contact lenses. For the imaging study, additional exclusionary criteria included: 18) over the age of 60; (19) metal in the body; (20) claustrophobia; and (21) weight greater than 250 lbs (due to the dimensions of the scanner). In addition, (22) bilinguals were excluded from the imaging study because the brain systems subserving tasks that rely on verbal stimuli differ between native English speakers and bilinguals (Proverbio *et al*., 2002). Compared with the behavioral study, the age range for the imaging study was truncated to 21-60 years to balance for age across groups and to exclude very old adults whose brain activation patterns would introduce a greater degree of heterogeneity into the study because of age differences in prefrontal cortex function (Spreng *et al*., 2010). All participants were classified by menopause stage based on criteria from the Stages of Reproductive Aging Workshop (STRAW) consensus statement (Soules *et al*., 2001) which defines menopause stage based on bleeding patterns. In light of evidence supporting a relationship between estrogen and working memory, we investigated menopause phase across *COMT* genotype and HIV serostatus groups as well as the interaction of genotype by serostatus. Menstrual cycle phase is not assessed during the standard, biannual WIHS visit.

2. *Behavioral Study*

One hundred and forty women (97 HIV-infected and 44 HIV-uninfected) between 25 and 71 year of age (M = 41.98, SD = 9.62) were enrolled in the study. WIHS participants were asked whether or not they wanted to complete the task and, if they did complete the task, they were paid ten dollars. In order to minimize staff burden, most exclusionary criteria, particularly those requiring review of medical records, were applied after participants completed the N-back. After completing the N-back, 34 of the 140 women were excluded from the study for the following exclusionary criterions: self-reported use of a stimulant drug in the past six months (n = 18); antipsychotic medication use (n = 4); serious head

injury (n = 4); stroke or cerebrovascular disease (n = 3); uncontrolled diabetes (n = 2); diagnosed with dementia (n = 1); AIDS-defining condition affecting the CNS (n = 1); and fewer than eight years of formal education (n = 1).

After applying exclusionary criteria, the study sample included 106 WIHS participants. An additional nine women (9.5%) were excluded from data analyses due to outlier behavioral performance as defined by performing below chance (less than 25% accuracy) or greater than three standard deviations from the overall mean on any N-back condition. Therefore, 97 participants were included in the final sample with 67 HIV-infected and 30 HIV-uninfected controls. Compared to the nine women excluded from data analysis, the 97 women included in the analysis reported less depressive symptoms ($p < 0.05$).

3. *Imaging Study*

The imaging study was nested in a parent study entitled, "Predictors of Brain Function in Women with HIV". The other primary study investigated the effects of drug use on verbal memory and hippocampal function in women with HIV. The parent study recruited and scanned 56 women from the WIHS study and inclusionary and exclusionary criteria were applied for both substudies prior to enrollment. Ten of the women in the parent study were not enrolled in the current study because they recently used drugs known to affect dopamine transmission (i.e. crack, cocaine and heroin). Twenty-nine HIV-infected women and 17 HIV-uninfected controls aged 27 to 59 (M = 42.6, SD = 8.5) years were enrolled in the study. Twenty-two of these women also participated in the behavioral study. Thirteen women were excluded from data analysis for the following reasons: technological difficulties during their scans leading to missing data (n = 4); a positive toxicology screen for recent illicit drug use (n = 1); and excess movement in the scanner which distorts the images (n = 6). Excess movement was defined as any movement during a scan that exceeded the size of two voxels or 6.25 mm. Lastly, two

women were excluded due to outlier behavioral performance as defined by a N-back percent accuracy score less than 25 percent or greater than three standard deviations from the overall mean on any condition. The 13 excluded participants did not differ from the 33 included participants on any demographic, behavioral or clinical variable (all *p*'s > 0.05). The final sample included 33 participants with 20 HIV-infected women and 13 HIV-uninfected controls.

B. *Materials*

1. *Measures*

a. *Center for Epidemiological Studies, Depression Scale (CES-D) (*Radloff, 1977*)*. The CES-D is a self-administered questionnaire designed to assess depressive symptoms in the general population. Participants were asked to report the frequency with which they experienced symptoms (e.g., "I felt that everything I did was an effort") over the past two weeks. Responses to 20 questions are given on a four-point Likert scale ("rarely" to "most of the time"). The total score ranges from 0 to 60 with higher scores indicating more depressive symptoms. The CES-D is administered to all WIHS participants at each of their biannual WIHS visits. Depressive symptoms are highly prevalent in the HIV population (Richardson *et al*., 2001) and, therefore, CES-D scores were systematically investigated across *COMT* genotype and HIV serostatus groups.

b. *Wide Range Achievement Oral Reading Test (WRAT) (*Jastak *et al*., 1984*)*. The WRAT is an achievement test that measures an individual's ability to read words. The test was included as a measure of educational attainment based on evidence that the WRAT and other literacy measures are a more valid index of educational experience than years of school among African Americans (Manly *et al.*, 2003). The raw score is the number of words that are correctly pronounced. We used a standard score which is the raw score adjusted for years of age. The standard score ranges between 45 and 124. The WRAT was administered to participants at one of the core WIHS visits

c. *The N-back Test* (Gevins & Cutillo, 1993). The N-back test is a well-validated test of working memory. The test involves three distinct conditions: 0-back, 1-back and 2-back. General instructions were read to the participant before initiating the task. Once the task was initiated, reminders to the instructions were presented prior to each condition. In all conditions, participants were required to monitor a series of sequentially presented letters (presentation duration 200 ms; 1.8 s inter-stimulus interval) on a computer screen. For the 0-back conditions, participants were instructed to retain in memory a target letter that had been presented in the instructions. If the currently presented letter matched the target letter, participants were instructed to press the "yes" button. If the currently presented letter did not match the target letter, participants were instructed to press the "no" button. The 0-back condition is used as the control condition because, although it involves short-term memory, it does not involve working memory and can be used as a measure of attention. In the 1-back condition, participants were instructed to press the "yes" button when the presented letter matched the letter on the previous trial. Participants were instructed to press the "no" button when the presented letter did not match the letter on the previous trial. Thus, participants continuously recalled an item that was "1 back" in a sequence. In the 2-back condition, participants were instructed to determine if the letter presented matched the letter presented two trials earlier and pressed the corresponding button. Compared to the 1-back, the 2-back condition involves a longer delay between stimulus presentation and recall and a greater information load to be held in working memory. The stimuli consist of pseudorandom sequences of letters with the number of "yes" button presses occurring in 25 percent of the trials. The participant received each N-back condition twice consecutively with 40 trials comprising each condition. The conditions were presented in order of difficulty (0-, 1-, 2-back). In the behavioral study, the N-back was administered on a laptop computer at the WIHS CORE Center in Chicago during one of the participants' biannual WIHS visits. The test was 10 minutes in length. The primary outcome measure was percent

accuracy (percent of correct responses) per condition and the secondary outcome was mean reaction time (RT) per condition.

d. *State and Trait Anxiety Inventory (STAI-6)* (Speilberger, 1993). The short form of the STAI is a self-report measure of six items measuring the extent to which the participant feels calm, tense, upset, relaxed, content, and worried. Ratings are made on a 4-point Likert scale ranging from "not at all" to "very much so." Scores range from 6 to 24, with higher scores correlating with greater anxiety.

e. *Genotyping assays*

1.) Behavioral & Imaging Studies: The *COMT* Val158Met genotype was collected on WIHS participants as part of a separate, genome wide association study in WIHS. Genotyping took place at Illumina Assay Services in San Diego, CA. Genotype data on a subset of 35 women were validated by genotyping conducted at the Biology of Addictive Diseases at Rockefeller University. The DNA sequence corresponding to the SNP to be analyzed was derived from the National Center for Biotechnology Information (NCBI) SNP Database (dbSNP) using the accession numbers, rs4680.

High-throughput DNA isolation was performed on immortalized B-cells and cell pellets from the WIHS participants who consented to participate in host genetic studies. The DNA isolation chemistry utilized silica-gel based DNA binding membranes in 96-well formats (Qiagen Inc., Valencia, CA). Purification of the DNA was performed using a robotic platform. The purified DNA was transferred into six individual bar code labeled screw cap tubes per subject and stored at –80C until use. Quantitation of DNA samples was performed using a PICO Green kit (Molecular Probes, Eugene, OR) and a SpectraMax Gemini XS fluorometer (Molecular Devices, Sunnyvale, CA).

Genotyping was completed using a combination of assay platforms. One platform involved employing previously validated commercially available assays (C__25746809_50) purchased from Applied Biosystems (www.appliedbiosystems.com) using the 5'-nuclease activity of Taq polymerase

and the use of fluorogenic probes (TaqMan) (Lee *et al*., 1993). In this assay two oligonucleotide probes specific for each of the alleles are labeled with different fluorophores (reporters) and included during the polymerase chain reaction (PCR) amplification. The presence of quencher fluorophores on the probes prevents fluorescence of the reporter dye while the oligonucleotide probe is intact (Livak *et al*., 1995). Hybridization of the reporter probe to its target sequence leads to nucleolytic cleavage of the probe during DNA synthesis as a result of the 5'->3' nuclease activity of Taq polymerase. Cleavage of the probe frees the reporter fluorophore from the quencher fluorophore and results in fluorescence that is proportional to the amount of target sequence present in the PCR (Livak *et al*., 1995). Fluorescence is detected using an ABI 7900HT sequence detection system and the alleles are scored using Sequence Detector software (ABI). The genotype of individual samples is accomplished within several minutes after PCR amplification is completed with no additional manipulation steps. In addition, the PCR tubes remain closed after amplification dramatically reducing any chance of contamination within the laboratory between samples and PCR runs.

The second platform involved the extraction of SNP content for the *COMT* Val158Met SNP featured on the Omni 2.5 SNP genotyping array (Illumina, Inc) which was recently employed in a genome-wide association study being conducted in the WIHS. This Infinium® assay leverages proven chemistry and a robust platform to produce superior data quality, high call rates, and consistent reproducibility. This array set provides comprehensive tagging SNP (tagSNP) data across the entire genome, capturing SNPs with minor allele frequencies as low as 1.0%. Benefiting from both HapMap (www.hapmap.org) and the 1000 Genomes Project (1kGP; www.1000genomes.org), the Omni 2.5 tool features SNPs with superior informativeness in all major racial and ethnic groups represented in the WIHS (i.e., African American, Hispanic, Asian, Caucasian). Arrays were processed according to the standard Illumina protocol. In brief, this involves whole genome amplification of 400 nanograms of

genomic DNA followed by DNA fragmentation in a single tube sample preparation without PCR or ligation steps, significantly reducing labor and sample handling errors. Then, allelic detection proceeds in two steps: first, unlabelled DNA fragments are hybridized to 50mer probes on the array to provide assay selectivity; second, enzymatic single-base extension with a labeled nucleotide provides assay specificity. Then, arrays are processed and imaged on the iScan Imaging System®. The genotype data will be imported into GenomeStudio for initial analyses (i.e., raw data normalization, clustering, and genotype calling).

Genotyping was performed blinded to the clinical status of participants; positive controls (i.e., intra-assay sample replicate, inter-assay sample replicate) were included to calculate quality control statistics. Given the fact that a standard quality control statistic, the Hardy-Weinberg test, is not reliable in a cohort composed of several racial and ethnic groups, an alternative strategy was employed where a random subset of 2% of the cohort was repeated and concordance statistics used as a measure of data QC. Any samples with replicate, intra-, or inter-assay concordance rates that fell below 95% were excluded. Then, samples were excluded that displayed poor genotyping success rate (<90%). Following the removal of those individuals not meeting this criterion, the second step was to exclude SNPs of poor quality (i.e., low call rate). SNP call rate is plotted for the remaining SNPs, where those SNPs falling below 95% were removed from further consideration. All SNPs that passed all quality control filters are retained for downstream analyses and the specific SNPs required for the current study were extracted for analysis.

2.) Imaging study: Val158Met genotype data was collected on most participants using the methods described above. Genotype data had not been previously collected on four imaging study participants because they were newly recruited to WIHS. Therefore, a saliva sample was collected on the four participants during their imaging study visit in order to conduct genotyping at the University of

Illinois at Chicago in the laboratory of our collaborator, Dr. Jeffrey Bishop. Saliva samples were collected using the Oragene-DNA self-collection kit OG-500 (DNA Genotek Inc., Kanata, Ontario, Canada). Participants spit into a saliva collection tube after not eating, drinking, smoking or chewing gum for 30 minutes. Once the saliva level reached a fill line, the lid of collection tube is closed, which causes the release of 2ml of preservation fluid from the cap into the saliva. This fluid allows for the purification and stabilization of DNA so that samples can be stored at room temperature before genotyping. Genomic DNA was extracted from saliva samples using the iPrep Pure Link gDNA Blood Kit on iPrep Purification Instrument (Invitrogen, Carlsbad CA).

After DNA isolation from a saliva sample, the Val158Met genotype was determined by PCR and Pyrosequencing analysis. The Pyrosequencing assay was designed by using PSQ Assay Design software (v 1.0.6) and validated with direct sequencing to ensure accuracy. The PCR was performed utilizing forward primer 5'-AGATCGTGGACGCCGTGA -3' and the biotinylated reverse primer 5'-AACGGGTCAGGCATGCAC -3'. Each PCR reaction (20µl) consisted of 10µl of Hot StarTaq Master Mix (Qiagen, Valencia, CA), 25pmol of each primer, 6µl of nuclease free water and 20-100ng of gDNA or whole genome amplified product Amplification was done in Eppendorf Mastercycler EP Gradient S Thermocycler (Eppendorf North America Inc., New York, NY) with denaturation for 15 minutes at 95°C, followed by 42 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 45 seconds, with a final extension of 72°C for 10 minutes.

Pyrosequencing was done using a PSQ HS96 Pyrosequencer (Biotage, Charlottesville, VA) and PSQ96 SNP software (v 1.2.1). For Pyrosequencing reactions, 5-7µl of the PCR product in a single well was immobilized with a mixture of 2µl streptavidin sepharose beads and 38µl of binding buffer by incubation at 1400rpm for at least 15 minutes and until ready for next step at room temperature. The primer plate (PSQ HS 96 plate) contained 0.3µM sequencing primer 5'-ATGGTGGATTTCGCT-3' in

12µl annealing buffer. Vacuum Prep workstation was used for strand separation. The beads containing immobilized templates were captured on filter probes and flushed through 70% ethanol for 5 seconds and then flushed through denaturation solution (0.2M NaOH) for 5 seconds followed by wash buffer rinse for 5 seconds. All liquid was completely drained from the probes and beads were released into a PSQ 96 plate containing the sequencing primer.

The PSQ 96 plate was heated at 80°C for 2 minutes, and then allowed to cool to room temperature. The final step of Pyrosequencing includes the addition of appropriate volumes of A, C, G, and T nucleotides. Prior to loading into the PSQ HS dispensing tips, each nucleotide is mixed with an equal volume of 1x TE Buffer. Enzymes and substrate quantities are also inserted into PSQ HS dispensing tips. PSQ HS dispensing tip holder was loaded into the Pyrosequencer.

C. *Procedure*

Behavioral study: Participants were recruited during the standard protocol for biannual WIHS visits. The current study was ancillary to the WIHS parent study and, therefore, there was no need for a separate consent form for this study. The general WIHS consent form includes the procedures of cognitive testing and genetic testing, although WIHS participants are offered the option of consenting to the WIHS while opting out of the genetic testing component. The N-back was given on a laptop computer as part of a one-time, cross-sectional, cognitive assessment that was incorporated into WIHS visits between May and November of 2008.

Imaging study: Research coordinators at the Chicago WIHS site determined which participants were initially eligible based on constant inclusionary/exclusionary criteria including age, years of education, and bilingualism. Women determined initially eligible for participation were given a short, verbal description of study procedures by WIHS research personnel at the end of their WIHS biannual visit. If an initially eligible woman was interested in participating, she either consented to have her

contact information passed along to study coordinators at UIC or she initiated contact by phone. UIC study coordinators screened the interested participants for the remaining exclusionary criteria and, if the participant met study qualifications, a study visit was scheduled. Throughout data collection, the UIC study coordinators were blinded to both participant genotype and HIV serostatus. The participants were also blinded to their own *COMT* genotype since the policy of WIHS as well as UIC state that genetic results cannot be disclosed to participants that are used strictly for research purposes. Currently, the *COMT* genotyping results are not important to one's health because there is no treatment recourses available based on the genetic information. This policy and the underlying reasoning were made clear to participants during the consenting process for WIHS and the current study.

Participants came to the Center for Magnetic Resonance (MR) Research at UIC for a two and a half hour study visit and transportation was provided, if necessary. After completing consenting procedures and signing a consent form, participants gave a small urine sample for an illicit drug toxicology screen and a pregnancy test. We conducted toxicology testing by way of a brief, in-house, litmus test (DrugCheck 5 panel cup by Express Diagnostics) in order to validate self-reported, recent use of illicit drugs including opiates, barbituates, tetrahydrocannabinol, methamphetamine, and cocaine. Study coordinators withdrew participants if the toxicology screen contradicted self-reported use of illicit drugs or the participant showed evidence of acute intoxication or withdraw. In order to rule out pregnancy, we conducted pregnancy tests using the hCG Urine Pregnancy Test Strip (Early-Pregnancy-Tests.com). Before scanning, participants completed a questionnaire assessing demographic and health-related outcomes and to verify that no changes had taken place in the participant's health since visit scheduling that would deem her ineligible for participation. Participants received detailed instructions on the N-back test and then completed a practice N-back on a laptop computer in order to familiarize them with the task. The practice N-back task mirrored the experimental task except that each condition

was given once and the number of trials was truncated to 20 trials per condition. Lastly, participants were scanned for prohibitive metal implants and taken to the scanner where they completed the STAI immediately prior to and following the scan in order to assess and control for anxiety symptoms while performing the scanner tasks. The N-back methodology was the same in the imaging study as the behavioral study except that the test was administered in the scanner and the number of trials per condition was truncated from 40 to 35 in order to allow for a manageable scanning time. The fMRI N-back was nine minutes in length.

Blood oxygen level-dependent (BOLD) imaging was performed on a General Electric 3.0 Tesla scanner. The fMRI N-back test was administered using a block design to optimize spatial localization of function. Thirty-seven images were acquired through the cerebral hemispheres in an oblique, axial plane. The acquisition parameters for the N-back were: TR = 2000ms, TE = 25 ms, flip angle 90°, NEX = 1, acquisition matrix = 64x64, FOV = 20 cm^2, slice thickness = 3mm, skip = 1 mm, slices = 37 axial, volumes=270. In addition, participants underwent routine structural MRI to provide anatomical data that was used for coregistration. MRI imaging was performed with a three-dimensional inversion recovery prepared spoiled gradient recalled echo (3D IRPrepSPGR) acquisition (reconstructed in axial and sagittal planes, TR = 13.8 ms, TE = 4.3 ms, flip angle = 25 degrees, acquisition matrix = 512x192, FOV = 22 cm^2, slices = 120, slice thickness = 1.5 mm, skip = 0 mm, NEX = 1, Bandwidth = 15.6 kHz, total acquisition time = 5:33 minutes). The scan session lasted one hour and involved other neurocognitive tasks and structural scans that were not part of the current study including a verbal memory task, a simple reaction time task, and diffusion tensor imaging.

Additional variables were collected as part of the CORE WIHS visit which was, on average, three months from the scanning date. During a standard WIHS visit, participants are asked about sociodemographic and health related variables including medication use, drug and alcohol consumption.

Markers of disease severity were also measured in HIV-infected women including CD4 absolute count (T helper cell count) and plasma HIV RNA viral load.

D. *Data Analysis*

Behavioral study: Five percent of participants were missing WRAT scores. Missing values were imputed using a regression based technique with race/ethnicity, age, and education, as predictors. The data was screened for outliers of the primary N-back outcome. An outlier was defined as a N-back percent accuracy score greater than three standard deviations from the overall mean on any condition

Previous *COMT* and working memory studies have reported significant genotype effects when contrasting Val/Val to the other combined genotype groups (i.e. Met/Met + Val/Met). Therefore, we chose to assess relationships between genotypes and neurocognitive outcomes assuming a dominant genetic model (i.e. Val/Val vs. Met allele carriers). We examined the main and interactive effects of HIV serostatus and *COMT* genotype on demographic and clinical characteristics using between-subjects ANOVAS for continuous variables and Chi-square (X^2) tests for categorical variables. To examine the independent and interactive effects of serostatus, *COMT* genotype, and N-back condition, a mixed factor ANOVA was conducted with N-back condition (0-, 1-, and 2-back) as the within-subjects factor and HIV serostatus (HIV+ vs. HIV-) and *COMT* genotype (Val/Val vs. Met allele carriers) as the between-subjects factor. Both an adjusted and unadjusted mixed factor ANOVA was conducted. Any demographic, behavioral or clinical variable that significantly differed between genotype or serostatus groups and significantly related to the main outcome (N-back) was included in the adjusted analyses as a covariate. All p values were two-sided with a statistical significance level of $p < 0.05$. All analyses were performed using SPSS statistical software (version 18.0 for Windows; SPSS, Chicago, IL).

fMRI study: The data analysis strategy used in the behavioral study was also applied to the behavioral data from the fMRI N-back test. Preprocessing and analysis of the imaging data was

completed using statistical parametric mapping (SPM5, Welcome Department of Imaging Neuroscience, London, UK) and Analysis of Functional Neuroimages (AFNI). Following acquisition, MRI data were converted to ANALYZE format using the DICOM conversion toolbox in SPM. The initial preprocessing step was motion correction and exclusion (> 6.25 mm of maximal displacement) using AFNI. Functional (i.e. EPI) images were realigned to the first volume of the task to correct for interscan movement and then co-registered to the individual's T1 structural volume. The structural image and co-registered functional images were spatially normalized to the SPM T1 MNI/ICBM (Montreal Neurologic Institute/International Consortium for Brain Mapping) template, and then smoothed with an 8 mm Gaussian kernel in order to minimize high frequency noise, thus increasing the signal-to-noise ratio. A first-level analysis step was conducted to determine regionally activated brain volumes in individual participants using a general linear model and convolved with the hemodynamic (BOLD) response function. In all imaging analyses, we used the subtraction method where brain activation associated with our control condition (0-back) is subtracted from the brain activation associated with the experimental conditions (1- and 2-back) (Peterson *et al*., 1989). The 0-back condition was used as a baseline comparison because the condition does not require the manipulation of information within working memory yet controls for visual attention and movement related activity. The assumption is that brain activation associated with attentional processes and motor planning and movement is removed and the remaining brain activation is associated with our cognitive process of interest, working memory. Therefore, two contrast images of brain activation were estimated for each participant and used in second-level fixed effects statistical models: 1) 1-back versus 0-back and 2) 2-back versus 0-back.

During a second-analysis step, we used the general linear model to determine significant activation associated with the interactive effects of genotype, serostatus and N-back condition. A threshold of $p < 0.01$ (uncorrected, minimum cluster size ≥ 15) was used when examining the two-way

interactive effects of genotype and serostatus and the three-way interactive effects of genotype, serostatus and N-back condition. A threshold of $p < 0.05$ was (uncorrected, minimum cluster size k = 15) was used when conducting follow-up tests to probe an interaction. We conducted a full factorial statistical model using the single-subject contrasts (1-back > 0-back; 2-back > 0-back) with the *COMT* genotype groups (Val/Val; Met allele carriers), HIV serostatus (HIV+; HIV-) and N-back condition (1-back; 2-back) as predictors. We conducted a region of interest analysis based on previous fMRI studies that found activation in these specific brain regions during N-back performance including the bilateral prefrontal and posterior parietal cortex, anterior cingulate, caudate and putamen (Bertolino *et al*., 2006; Callicott *et al*., 2000; Egan *et al*., 2001; Heinz and Smolka, 2006; Mattay *et al.*, 2003; Meyer-Lindenberg *et al*., 2006; Jacobs and D'Esposito, 2011). The resulting activation maps represented the probability of a brain region to show differences in activation levels (BOLD signal intensity) contingent upon HIV serostatus and *COMT* genotype. Anatomical localization of significant activations of interest was determined by converting MNI coordinates to standard Talairach coordinates using the Wakeforest pickatlas (Maldjian *et al*., 2003; Maldjian *et al*., 2004) and through use of standard atlases (Talairach and Tournoux, 1988) and the Talairach Client (Lancaster, 2000).

I. RESULTS

A. *Behavioral Study*

1. Demographics

Table 1 shows the demographic, behavioral and clinical information for the 97 participants by *COMT* genotype (55 Met carriers, 42 Val/Val) and HIV serostatus (67 HIV+, 30 HIV-). Overall, participants ranged in age from 25 to 71 years (M=41, SD=10). Minority representation was high, with 81% non-Hispanic African American and 12% non-African American, Hispanic participants. Among

HIV-infected women, 13% had a current CD4+ lymphocyte count less than 200 cells/μl and 58% had an undetectable plasma HIV RNA level (lower limit of detection was 80 copies per ml) indicating optimal treatment response. Seventy percent had been prescribed antiretroviral therapy, and, of those prescribed therapy, 83% reported treatment adherence as defined in WIHS as 95% of prescribed doses.

Compared with HIV-uninfected women, HIV-infected women were older (38.0 years vs.43.5 years) and had a higher prevalence of hepatitis C (10% vs. 36%) and past illicit drug use (i.e. crack, cocaine and heroin) (40% vs. 64%), $F(1, 93) = 14.34$, $p < 0.001$, $X^2 = 6.87$, $p < 0.01$, $X^2 = 4.93$, $p < 0.05$. Compared to Val/Val genotype carriers, Met allele carriers were more likely to have recently (past six months) used marijuana (5% vs. 20%), $X^2 = 4.76$, $p < 0.05$. There was a significant genotype by serostatus interaction on education, menopause stage, rates of hazardous drinking (greater than seven drinks per week) and recent marijuana use. Specifically, among Met allele carriers only, HIV-uninfected women had a significantly higher proportion of high school graduates (91% vs. 58%) and premenopausal women (80% vs. 34%) and a higher prevalence of hazardous drinking (36% vs. 4%) and recent (past six months) marijuana use (45% vs. 13%) compared to HIV-infected women, $X^2 = 3.94$, $p < 0.05$, $X^2 = 6.92$, $p < 0.05$, $X^2 = 9.17$, $p < 0.01$, $X^2 = 5.57$, $p < 0.05$. Any demographic, behavioral or clinical variable that significantly differed between genotype or serostatus groups and significantly related to the main outcome (N-back) was included in the adjusted analyses as a covariate. Past illicit drug use (i.e. crack, cocaine, and heroin) was included as a covariate given serostatus differences in this factor and its significant association with percent accuracy on all N-back conditions, all *p*s < 0.05.

Genotype distributions were in Hardy-Weinberg equilibrium for the full sample, $X^2 = 0.6$, $p > 0.05$, and within the predominant race group, African Americans, $X^2 = 0.08$, $p > 0.05$. Table 2 provides the *COMT* Val158Met genotype distribution by HIV serostatus groups for the behavioral study. Although it did not reach significance, the distribution of *COMT* genotype differed by serostatus where

the proportion of Met allele carriers was higher in HIV-infected women (67%) compared to HIV-uninfected women (37%), $X^2 = 2.3$, $p = 0.13$.

2. Does HIV serostatus and COMT genotype impact working memory performance?

a. Unadjusted Analysis

The parametric effects of working memory load across the N-back task were reflected in performance. As expected, there was a significant main effect of N-back condition on percent accuracy, F (2, 186) = 57.98, $p < 0.001$, where accuracy was significantly greater on the 1-back versus the 0-back, F (1, 93) = 45.43, $p < 0.001$ and on the 2-back versus the 1-back, F (1, 93) = 15.31, $p < 0.001$. The increasing task difficulty was further supported by a significant main effect of N-back condition on reaction time, F (2, 186) = 19.56, $p < 0.001$, and this effect was driven by the significant increase in reaction time from the 0- to 1-back F (1, 92) = 5.79, $p < 0.05$, but not from the 1- to 2-back, $p > 0.05$. Unexpectedly, there was no main effect of *COMT* genotype on N-back performance, $p > 0.05$; however, consistent with hypotheses, N-back percent accuracy was significantly higher in the HIV-uninfected controls ($M = 76.3$, $SE = 2.2$) compared to the HIV-infected group ($M = 68.7$, $SE = 1.5$), F (1, 93) = 7.94, $p < 0.01$. Furthermore, there was a significant serostatus by genotype interaction on N-back percent accuracy, F (1, 93) = 7.34, $p < 0.01$. Given that there was a disproportionate genotype distribution whereby Met allele carriers were the majority in HIV-infected (67%) women and the minority in HIV-uninfected women (37%), we probed the interaction by examining the effect of serostatus within each genotype group. Follow-up tests indicated that the negative effect of HIV on N-back scores was only observed among Val/Val carriers, F (1, 40) = 51.77, $p < 0.001$, and not Met allele carriers, $p > 0.05$. There was no three-way interaction among N-back condition, serostatus and genotype suggesting that N-back condition did not moderate the interaction of serostatus and genotype and there were no interactive effects on N-back reaction time (all ps > 0.05).

b. Adjusted Analysis

After adjusting for past illicit drug use, the effects of N-back condition, serostatus and the interaction of genotype and serostatus on percent accuracy remained significant. The significant main effect of N-back condition on percent accuracy, F (2, 184) = 3.48, $p < 0.05$, was driven by the significant difference between 0- and 1-back F (1, 92) = 5.79, $p < 0.05$, but not between 1-back and 2-back scores, $p > 0.05$. Conversely, the effect of N-back condition on reaction time did not persist after adjusting for past illicit drug use, $p < 0.05$. Consistent with hypotheses and the unadjusted analysis, the main effect of HIV serostatus indicated that HIV-infected women (M = 69.3, SE = 1.5) had a significantly lower N-back percent accuracy, across all N-back conditions, compared to HIV-uninfected women (M = 75.3, SE = 2.2), F (1, 92) = 5.09, $p < 0.05$. Inconsistent with hypothesis yet consistent with the unadjusted analysis, the Val/Val genotype did not negatively impact working memory performance across serostatus groups and, therefore, the Val/Val genotype did not compound the negative effects of HIV. Rather, similar to the unadjusted analysis, there was a significant HIV serostatus by genotype interaction on N-back performance, F (1, 92) = 7.16, $p < 0.01$ that revealed a significant HIV serostatus effect among Val/Val but not Met allele carriers. More specifically, among Val/Val carriers, N-back accuracy was significantly lower across all conditions in HIV-infected women (M = 67.2, SE = 2.5) versus uninfected women (M = 80.3, SE = 2.8), F (1, 39) = 12.96, $p < 0.001$. Conversely, among Met allele carriers, N-back performance did not differ between HIV-infected and uninfected women, $p > 0.05$ (see Figure 2). There was no three-way interaction among N-back condition, serostatus and genotype suggesting that N-back condition did not moderate the interaction of serostatus and genotype. Lastly, there were no independent or interactive effects of serostatus and genotype on N-back reaction time, all ps > 0.05 (see Figure 5).

B. *Imaging Study*

1. *Demographics*

Table 3 shows the demographic, behavioral and clinical information for the 33 participants by *COMT* genotype (18 Met carriers, 15 Val/Val) and HIV serostatus (20 HIV+, 13 HIV-). Participants ranged in age from 27 to 58 years (M = 43, SD = 8). Minority representation was high with 94% African-American participants. Among HIV-infected women, 5% had a current CD4+ lymphocyte count less than 200 cells/μl and 60% had an undetectable plasma HIV RNA level (lower limit of detection was 80 copies per ml) indicating optimal treatment response. Eighty-five percent had been prescribed cART and of those prescribed cART, 82% reported treatment adherence for greater than 95% of prescribed doses.

There was no significant effect of serostatus, genotype or the interaction of serostatus and genotype on any of the demographic, behavioral or clinical variables. Recent marijuana use differed between genotype groups at the level of a statistical trend $X^2 = 3.53$, $p = 0.06$, whereby Met allele carriers tended to have a greater proportion of recent users compared to Val/Val carriers. We found no differences in STAI scores from pre- to post-scan in the full sample or by genotype or by serostatus groups, all ps > 0.05. The mean score was 8.6 (range: 6 to 24) for the pre-scan STAI assessment and 8.4 for the post-scan STAI indicating that behavioral performance and brain activation patterns were not impacted by the presence of anxiety symptoms during the scan. There were no group differences in demographic, clinical or behavioral variables, and none of those variables had a significant or near significant association with N-back percent accuracy. Therefore, we did not adjust for covariates in statistical analyses.

Genotype distributions were in Hardy-Weinberg equilibrium for the entire sample, $X^2 = 0.07$, $p > 0.05$, and within the predominant race group, African Americans, $X^2 = 0.29$, $p > 0.05$. Table 2 provides the *COMT* Val158Met genotype distribution by HIV serostatus groups. Similar to the behavioral study,

the distribution of *COMT* genotype differed by HIV serostatus; however, in the imaging sample, this difference was statistically significant rather than a trend. The proportion of Met allele carriers was significantly higher in HIV-infected women (65%) compared to HIV-uninfected women (38%), X^2 = 7.9, p = 0.005. Therefore, the HIV-infected women were more likely to have the typically beneficial genotype or the Met allele, and the HIV-uninfected controls were more likely to have the typically detrimental genotype or Val/Val. The result is a statistical confound that reduces the likelihood of detecting the overall effects of HIV and genotype. For this reason we examined the effect of serostatus and the interaction between serostatus and N-back at the level of Val/Val and at the level of Met. In order to see if the genotype distribution pattern was unique to this study sample or representative of women with HIV, we examined the effect of serostatus on the *COMT* Val158Met genotype distribution in the WIHS-wide cohort (n = 2,087). Similar to our study sample, the distribution of *COMT* genotype significantly differed by serostatus group, X^2 = 10.31, $p < 0.01$, whereby the proportion of Met allele carriers was significantly higher in HIV-infected women (63%) compared to HIV-uninfected women (55%). Therefore, the *COMT* genotype distribution in our study sample is representative of women with HIV in the United States.

2. *Does HIV serostatus and COMT genotype impact working memory performance?*

There was a significant main effect of N-back condition on N-back percent accuracy, F (2, 58) = 59.09, $p < 0.001$. Accuracy was significantly greater on the 0-back versus the 1-back, F (1, 32) = 35.40, $p < 0.001$ and on the 1-back versus the 2-back, F (1, 32) = 37.0, $p < 0.001$. Furthermore, there was a significant main effect of N-back condition on reaction time, F (2, 58) = 57.83, $p < 0.001$, whereby, reaction time is significantly higher in the 1-back versus the 0-back, F (1, 29) = 39.19, $p <$ 0.001, and in the 2-back versus the 1-back, F (1, 29) = 24.00, $p < 0.001$. In contrast to the behavioral study, serostatus and *COMT* genotype had no independent or interactive effects on N-back percent

accuracy, all *p*s > 0.05. Similar to the behavioral study, there were no independent or interactive effects of serostatus and genotype on N-back reaction time, all *p*s > 0.05.

3. *Does HIV serostatus and COMT genotype impact brain activation patterns during the N-back?*

In order to examine the neural correlates underlying the genotype by serostatus interaction, we examined BOLD activity in *a priori* brain areas using a region of interest approach. To determine whether our controls showed the expected, typical patterns of activation during the n-back, we compared brain activation in the HIV-uninfected controls to brain activation previously reported in healthy control populations during the N-back task. Consistent with previous reports (Bertolino *et al*., 2006; Callicott *et al*., 2000; Heinz & Smolka, 2006; Jacobs & D'Esposito, 2011; Mattay *et al*., 2003; Meyer-Lindenberg *et al*., 2006), we observed significant BOLD responses in the working memory cortical network including right anterior cingulate (Brodmann Area [BA] 32), bilateral dorsolateral prefrontal cortex (DLPFC) (BA 8/BA 9/BA 46), bilateral ventrolateral prefrontal cortex (BA 45/47), right superior frontal gyrus (BA 8), and bilateral posterior parietal cortex (BA 7). Figure 3 provides a visual depiction of the brain activation associated with the N-back test in HIV-uninfected controls.

In demonstration of the parametric effects of the fMRI N-back task, there was a significant main effect of N-back condition on brain activation across serostatus groups. More specifically, we found an association between an increase in working memory load from the 1- to 2-back and increased BOLD signal in the bilateral posterior parietal lobe (BA 19, BA 39, BA 7), bilateral DLPFC (BA 9), bilateral superior frontal gyrus (BA 8), and the left inferior frontal gyrus (BA 44). Table 5 provides the Talairach coordinates and statistical values associated with each brain region showing significantly greater activation in the 2-back versus the 1-back.

When examining the interaction of serostatus by genotype on the BOLD response associated with working memory, significant BOLD signal was found in the left anterior cingulate (BA 24, k = 66),

right anterior cingulate (BA 32, BA 25), right superior frontal gyrus (BA 10), and right middle frontal gyrus (BA 11, BA 10). Table 6 provides the Talairach coordinates and statistical values for each significantly active brain region in the serostatus by genotype interaction. We probed the interaction by examining the effect of HIV serostatus on brain activation patterns in the working memory network within each genotype group separately (Val/Val vs. Met allele carriers). Within Val/Val carriers, results were consistent with previous fMRI studies investigating brain response to working memory in HIV in that we found significantly greater frontal activation in the HIV-infected individuals compared to uninfected controls. More specifically, HIV-infected individuals demonstrated significantly greater activation in the left anterior cingulate (BA 24), right anterior cingulate (BA 32, BA 25), right DLPFC (BA10), and right medial frontal gyrus (BA 10) (see Figure 4). Conversely, within Met allele carriers, HIV-infected individuals did not demonstrate significantly greater brain activation in comparison to uninfected controls. Rather, HIV-uninfected controls showed significantly greater activation in the right medial frontal gyrus (BA 11) and the left anterior cingulate (BA 24) compared to the HIV-infected individuals (see Figure 5).

The three-way interaction of HIV serostatus by genotype by N-back condition was associated with significant brain activation in the right anterior cingulate (BA 32, k = 174, $z = 4.12$) and left medial frontal gyrus (BA 9, k = 15, $z = 3.35$) indicating that N-back condition moderates the interactive effects of genotype and serostatus on brain activation. These significant clusters of activation were driven by the increased activation in the HIV-infected groups versus the seronegative controls among Val/Val carriers in the 2-back condition. Table 7 provides the Talairach coordinates and statistical values associated with significantly active brain regions associated with the serostatus by genotype by N-back condition interaction.

II. DISCUSSION

Previous studies demonstrated associations among HIV infection, dopamine dysfunction, and working memory deficits, but this study is the first to examine the interactive effects of *COMT* genotype and HIV serostatus on cognition. Thus far, two studies have examined the effect of *COMT* genotype on executive function in HIV-infected individuals but those studies did not include HIV-uninfected controls (Bousman et al., 2011; Levine et al., 2011). Levine et al. reported no effect of *COMT* genotype on executive function in HIV (2011). Conversely, Bousman *et al.* (2010) found an association between the Met/Met genotype and better executive function among HIV-infected men who were not methamphetamine users. Our inclusion of HIV-uninfected participants allowed for the assessment of whether genotype moderates the effect of serostatus on cognition. Our finding of a working memory deficit in HIV supported our hypothesis and replicated previous findings (Hinkin *et al*., 2002; Stout *et al*., 1995; Sun *et al*., 2010); however, inconsistent with hypotheses, we did not find a significant main effect of *COMT* genotype on working memory. Our ability to detect an effect of *COMT* may have been undermined by our disproportionate genotype distribution where the beneficial Met allele was more prevalent among HIV-infected women and the detrimental Val/Val genotype was more prevalent among HIV-uninfected women. Additionally, the genotype distribution precluded us from testing our hypothesis that the relationship between the Val/Val gene and poorer working memory performance would not differ between HIV-infected and uninfected women. Rather, we probed the genotype by serostatus interaction by examining the effect of serostatus within each genotype group in order to better equate the sample size across follow-up tests. This analysis revealed a novel finding that that the effect of HIV is dependent on *COMT* genotype in that the negative impact of HIV infection on working memory was due to Val/Val genotype carriers. We predicted that the Val/Val genotype would compound the negative effects of HIV so that HIV-infected women with the Val/Val genotype would

show the worst performance. We did find that HIV-infected, Val/Val carriers showed the worst performance; however, the Val/Val genotype did not compound the negative effect of HIV on working memory. Rather, the negative effect of HIV was driven by individuals with the Val/Val genotype and the associated suboptimal prefrontal dopamine levels. The negative effect of HIV infection across the full sample and within Val/Val carriers was consistent across all N-back conditions (0-, 1- and 2-back) indicating that HIV impacts all components of working memory including attention, short-term memory and the updating and temporal indexing of incoming stimuli. This impact is present even at lower levels of stimuli load and delay (1-back).

Among Met allele carriers, there was no difference between HIV serostatus groups on working memory performance. This suggests that there is a threshold level of hypodopaminergic signaling that must be surpassed in order for the effects of HIV to manifest. Perhaps the combination of the Val/Val genotype and HIV surpasses this threshold leading to working memory deficits. When considering the inverted U-shaped curve that characterizes the relationship between executive function performance and prefrontal dopamine levels (Cai and Arnsten, 1997; Egan *et al*., 2001; Granon *et al*., 2000; Zahrt *et al*., 1997), the combination of the Val/Val and HIV infection appears to push prefrontal dopamine levels far enough to the left of the curve that executive function becomes compromised to a degree where deficits are observable.

Previous studies have consistently found a negative impact of HIV on working memory (Hinkin *et al*., 2002; Stout *et al*., 1995; Sun *et al*., 2010). Although the Val/Val genotype was not the predominant genotype within our sample of HIV-infected women, the Val/Val genotype is the ancestral, more common, genotype. Depending on ethnicity with estimates in the upper range for African Americans, the frequency of the Val/Val genotype is estimated between 58 to 69 percent according to data from the International HapMap Project and a previous study (Beuten et al., 2006).

Therefore, the consistency of the finding of an HIV-associated executive function deficit could be attributed to the assumption that the majority of study samples are HIV-infected individuals with the Val/Val genotype or suboptimal, prefrontal dopamine levels. Our study underscores the importance of assessing and controlling for genetic polymorphisms that influence prefrontal dopamine function in order to accurately assess the effects of HIV on prefrontal-mediated functions.

As previously cited, Bousman *et al.* (2010) found an association between the Met/Met genotype and better executive function among men with and without HIV who were not methamphetamine users. These findings are concordant with ours in that the Met allele demonstrated a beneficial effect to working memory in HIV. Conversely, our findings are discordant with Levine and colleagues (2011) who reported no association between the Val158Met SNP and working memory in a sample of 184 men and women with HIV. Methodological differences may help to explain the discordant results between the current study and those reported by Levine *et al*. (2011). For example, our sample consisted of mostly asymptomatic, HIV-infected individuals with a mean CD4 count of 511 (*SD* = 334) and a small proportion (13%) of participants with a CD4 count less than 200. Conversely, Levine *et al.* (2012) examined HIV-infected individuals who were in more advanced stages of illness as exemplified by their mean CD4 count of 219 (*SD* = 227). It is possible that, in the later stages of disease, the effects of HIV on working memory become more robust so that any genotype effect is masked. Secondly, our measure of working memory differed in that we used the N-back test and Levine *et al*. (2012) used the Letter-Number Sequencing test and the PASAT. Perhaps the relationship between *COMT* and executive function is test-specific. We chose the N-back test because it has consistently shown effects across *COMT* genotype groups. However, other working memory tests may be less sensitive to the effects of *COMT* depending on difficulty level and the cognitive processes involved. Lastly, sex differences are present between the two studies in that we solely examined women, whereas, women

only comprised thirteen percent of the sample in the Levine *et al.* (2012) study. Levine *et al*. (2012) did not examine the interaction between *COMT* genotype and sex, and they did not find that sex was a significant predictor of neurocognitive performance; however, they may have been underpowered given the small percent of women in their sample. Sex is likely influential in the effects of *COMT* given evidence from animal studies that estradiol augments dopamine activity (Becker, 2000; Thompson and Moss, 1994; Xiao and Becker, 1994). Consequently, where one's PFC dopamine levels fall on the inverted U-shaped curve is likely to be partially dependent on endogenous estradiol levels. This is supported by findings from Jacobs and D'Esposito (2011) that the effect of endogenous estradiol fluctuations on working memory performance in healthy, young women is dependent on *COMT* Val158Met genotype.

The aim of the imaging study was to investigate the neural correlates of the effect of *COMT* on working memory performance in women with HIV. In the imaging sample, the significant difference in *COMT* genotype distribution between serostatus groups presented a statistical confound that reduces the likelihood of detecting the overall effects of HIV and genotype. The HIV-infected women were more likely to have the typically beneficial genotype or the Met allele and the HIV-uninfected controls were more likely to have the typically detrimental genotype or Val/Val. Therefore, we focused our analysis on the interactive effect of serostatus and genotype and the interaction between serostatus and N-back at the level of Val/Val carriers and at the level of Met allele carriers. Notably, we found that the significant difference in genotype distribution by serostatus was representative of the WIHS-wide cohort. We speculate that the genotype difference by serostatus may be due to evidence of an association between the Met allele and greater risk-taking propensity (Amstadter *et al*., 2012; van den Bos *et al*., 2009) and risky sexual behavior in the context of methamphetamine use (Bousman *et al*.,

2010). Perhaps the presence of the Met allele and its associated personality characteristics puts individuals at a higher risk for contracting HIV due to risky behaviors.

Similar to the behavioral study, the serostatus by genotype interaction was significant indicating that the significant impact of *COMT* genotype on cortical, particularly prefrontal, function was contingent upon serostatus. In contrast to the behavioral study, we found a three-way interaction among serostatus, genotype and N-back condition indicating that the interactive effects of *COMT* and HIV on brain function are contingent upon the level of task difficulty. When solely examining Val/Val genotype carriers, our finding of greater brain activation in HIV-infected women versus uninfected controls replicates previous reports of an association between HIV and increased brain activity during working memory (Chang *et a*l., 2001; Ernst *et al*., 2002) and suggests that brain processing inefficiency contributes to the working memory deficit demonstrated by HIV-infected, Val/Val carriers. The specific brain regions that showed significantly greater activation in HIV-infected, Val/Val carriers included the anterior cingulate cortex, DLPFC and the medial PFC, which are all brain regions shown in previous studies examining brain activation differences in relation to HIV or *COMT* during working memory (Bertolino et al., 2006; Callicott *et al*., 2000; Egan *et al*., 2001; Heinz & Smolka, 2006; Jacobs & D'Esposito, 2011; Mattay *et al*., 2003; Meyer-Lindenberg *et al*., 2006).

Neuroimaging studies have implicated the anterior cingulate cortex in tasks involving multiple demands including attention, error detection and decision making, suggesting that this region helps to coordinate multiple cognitive processes associated with a challenging task (Chein and Schneider, 2005; Kondo *et al*., 2004; Peterson *et al*., 1999; Posner and DiGirolamo, 1998). An event-related, fMRI study reported that the error detection involves engagement of the anterior cingulate cortex during both correct and incorrect responses and the activation of the anterior cingulate increases as task difficulty increases (Carter *et al*., 1998). The N-back task involves multiple cognitive processes including attention, motor

planning and movement, short-term memory and the updating and temporal indexing of incoming stimuli. Therefore, the finding that the anterior cingulate showed significantly greater activation as N-back difficulty increased was anticipated. Furthermore, the idea that the engagement of the anterior cingulate is dependent upon *COMT* genotype and HIV serostatus is not surprising given that the anterior cingulate is well-connected with the PFC (Posner & DiGirolamo, 1998) and richly innervated by dopamine projections from the midbrain area (Seamans and Yang, 2004) Therefore, it is likely that the anterior cingulate is vulnerable to changes in dopamine transmission as determined by clinical status and genotype and this vulnerability increases with task difficulty.

Evidence suggests that the bilateral medial PFC regulates uncertainty (Elliot *et al*., 1999; Fukui *et al*., 2005; Volz *et al*., 2003; Volz *et al*., 2004). Significant activation in the medial PFC has been found in relation to an increase in guessing demands in a simple two-choice guessing task (Elliot *et al*., 1999) and to risky decision component of a decision-making task, the Iowa gambling task (Fukui et al., 2005). Volz and colleagues (2003) investigated the neural substrates of prediction under varying levels of uncertainty and found that increasing uncertainty was associated with greater activation in the medial PFC as well as subcortical areas known to regulate dopaminergic function. It is likely that the N-back task invokes a substantial degree of uncertainty, especially the 2-back condition, given that participants are instructed to respond to each individual trial even if they are unsure of the answer. Lastly, the DLPFC is the brain region most commonly cited in neuroimaging studies examining *COMT* and working memory, likely due the role of the DLPFC in multiple working memory processes including the monitoring and manipulation of information held in working memory (Owen, 1997; Petrides, 1994), response selection (Rowe *et al*., 2000), and strategy use (Bor *et al*., 2003, 2004) including the organization of information to enhance encoding (Fletcher *et al*., 1998). Tan *et al.* (2007) used an event-related fMRI design to investigate the neural underpinnings of the effects of *COMT* on specific working

memory processes and found that as the number of Val alleles increased, DLPFC activation amplified during the encoding of new information and the temporal integration of that information into working memory, but not at information retrieval. This suggests that the dopaminergic signaling in the DLPFC impacts the working memory processes involved in strategic encoding and not the retrieval of previously encoded information (Tan *et al.*, 2007).

When examining the effect of working memory load on brain activation, we found that the posterior parietal lobe, the DLPFC the superior frontal gyrus, and the left inferior frontal gyrus (BA 44) was significantly more active in the 2-back condition compared to the 1-back condition. This suggests that these specific brain regions are only engaged as task difficulty and working memory load increase. There is a degree of similarity between the network of active brain regions associated with the 2-back versus 1-back contrast and the brain regions that showed greater activation in HIV-infected versus uninfected Val/Val carriers. Both contrasts showed significant activation in the left anterior cingulate, the right DLPFC and the bilateral, posterior parietal cortex. In addition, the three-way interaction of serostatus by genotype by N-back condition appeared to be driven by the Val/Val genotype carriers in that the difference in activation observed in HIV-infected versus uninfected women was greater in the 2-back versus the 1-back condition. The modulatory effect of N-back condition among Val/Val carriers indicates that increased activation in HIV-infected versus uninfected women is more prominent when cognitive demand increases.

The concordance in brain activation patterns between the effect of N-back condition and the effect of serostatus within ValVal genotype carriers suggests that the increased activation in HIV-infected, Val/Val genotype carriers represents greater effort and recruitment of a larger network of brain areas in order to maintain behavioral competency. Taken from another perspective, the neural basis underlying the working memory deficit in HIV-infected, Val/Val carriers is proposed to be inefficient

brain processing, which is reflected by the compensatory recruitment of additional neural resources. The demonstration of inefficient prefrontal function in HIV-infected, Val/Val genotype carriers is consistent with previous studies citing prefrontal dysfunction as a core manifestation of HAND (Castelo *et al*., 2006; Chang *et al*., 2001; Ernst *et al.* 2002; Pfefferbaum *et al*., 2009; Woods *et al*., 2009) Electrophysiological and neural modeling studies suggest that inefficient prefrontal processing is due to a reduction in the signal-to-noise ratio in prefrontal neurons as a result of decreases in synaptic dopamine (Sawaguchi *et al*., 1990; Servan-Schreiber *et al*., 1990; Winterer and Goldman, 2003). More specifically, evidence suggests that a decrease in synaptic dopamine leads to a reduction in the signal-to-noise ratio by means of by modulating NMDA, non-NMDA, and GABAergic transmission and, in turn, de-stabilizing the prefrontal cortical networks (Seamans and Yang, 2004).

In contrast, the brain regions that showed greater activation in HIV-uninfected versus infected Met allele carriers did not correspond to brain areas related to working memory load. This suggests that the increased activation in HIV-uninfected versus infected Met allele carriers is not reflective of increased effort but represents a different network of neural resources with a function that is not representative of a brain response to increased task difficulty. It is difficult to speculate beyond this given that only five participants comprise the cell of HIV-uninfected, Met allele carriers. Further research with larger sample sizes and more direct measures of *COMT* enzyme activity could help to elucidate the finding of increased brain activation in HIV-uninfected versus infected Met allele carriers.

Although our findings provide novel insights into the interactive effects of *COMT* and HIV, certain limitations should be considered in regard to study design. Our sample size in the behavioral study is similar to other studies investigating *COMT* in relation to cognition; however, the specific cell of HIV-uninfected, Met allele carriers is small (n=11) and this should be taken into consideration when interpreting the results. Both the imaging and behavioral study samples revealed a disproportionate

distribution of *COMT* genotype between serostatus groups that was statistically significant in the imaging sample and, therefore, resulted in a statistical confound that prevented us from examining the main effects of HIV and *COMT* genotype on brain activation. In both the behavioral and imaging studies, the genotype distribution dictated our method of probing the genotype by serostatus interaction by examining the effect of serostatus within each genotype group in order to better equate the sample size across follow-up tests.

Menopause stage was assessed but did not relate to N-back scores and, consequently, was not entered as a covariate in data analysis. Menstrual cycle phase was not assessed in participants. Therefore, despite previous findings of an interactive relationship between *COMT* and estradiol (Jacobs and D'Esposito, 2011), we were not fully capable of controlling for endogenous estradiol levels. It can be assumed our study sample represented a range of estradiol levels and; therefore, generalizability is improved at the expense of statistical control. Given that the *COMT* enzyme metabolizes catecholestrogens and estrogen's agonistic effect on dopamine, we cannot exclude the possibility that estradiol signaling modulates the neural mechanism underlying the effects of *COMT*. Additionally, we relied solely on self-reported use of crack, cocaine or heroin in order to exclude individuals who used illicit drugs within the past six months from the behavioral study. We excluded recent drug users to avoid the confound of drug use and to generate more easily interpretable results; however, we cannot rule out the possibility of drug use confounding our results given the high rates of illicit drug use in the HIV population and our reliance on self-report. Another limitation to consider is that the *COMT* enzyme is not the sole mechanism by which dopamine signaling is regulated. The monoamine oxidase enzyme (MOA) and the dopamine transporter (DAT) also contribute to dopamine metabolism and there is genetic variation in the genes coding for the MOA and DAT enzymes that may also contribute to individual differences in PFC dopamine levels. Nevertheless, *COMT* seems to play a central role in

dopamine metabolism specifically in the PFC. The DAT is significantly less prevalent in the PFC in comparison to other brain areas such as the striatum where it is responsible for the majority of dopamine metabolism (Sesack et al. 1998). Dopamine regulation in the PFC is unique compared to the rest of the brain in that the *COMT* enzyme is responsible for more than 60 percent of dopamine degradation (Karoum et al. 1994). Therefore, the pivotal role of the *COMT* in PFC dopamine signaling in conjunction with the strong impact of the Val158Met SNP on *COMT* enzyme levels suggests that the Val158Met SNP is the primary contributing gene variant to PFC-mediated cognition.

The major strength of this study is that we are the first to investigate the interactive effects of *COMT* genotype and HIV on working memory performance and prefrontal function. The significance of this investigation stems from evidence that both HIV and *COMT* genotype impact dopaminergic signaling in the PFC which plays a central role in working memory. Findings highlight dopamine dysfunction as a neural mechanism underlying HIV-neurocognitive disorders and suggest that the decrease in dopaminergic function that results from the combination of HIV and the Val/Val genotype contributes to a vulnerability to working memory impairment and inefficient processing in the PFC. These results are novel and, therefore, necessitate replication in HIV-infected women and investigation in HIV-infected men.

A future, haplotype study would extend current findings of an impact of *COMT* in HIV to a systems-level interaction between neural networks underlying working memory. Evidence indicates that the *COMT* genotype shows an interactive effect on working memory and prefrontal function with other genetic polymorphisms implicated in working memory and/or dopamine signaling including variants on genes coding for glucocorticoid receptors (El-Hage *et al*., 2011) and the dopamine transporter (Bertolino *et al*., 2006) in healthy individuals. Examining these gene-gene interactions in HIV would help to further characterize the effect of *COMT* genotype in this population. Currently,

investigating genetic markers such as the *COMT* Val158Met SNP is an initial step in more thoroughly characterizing predictors of cognitive deficits in women with HIV. By doing this, we move a step closer to using biomarkers to help us target and personalize treatment for patients with HIV. *COMT* inhibitors such as tolcapone are under evaluation as a potential treatment strategy to improve dopaminergic function in hypodopaminergic clinical conditions (Apud *et al*, 2007; Farrell *et al*., 2012; Giakoumaki *et al*., 2008). A neuroimaging study by Apud *et al*. (2007) investigated the effect of tolcapone on prefrontal function in healthy individuals carrying the *COMT* Val/Val or Met/Met genotypes. They reported a treatment by *COMT* genotype interaction whereby tolcapone improved working memory and prefrontal processing efficiency in Val/Val, but not Met/Met genotype carriers. Similar to animal studies (Liljequist *et al*., 1997), the results demonstrate the potential therapeutic effect of *COMT* inhibitors on PFC function in states of lower dopaminergic transmission (Apud *et al*, 2007).

In conclusion, *COMT* genotype appears to moderate the effect of HIV on working memory performance and physiological response in the anterior cingulate and PFC. Results suggest that genetically-determined dopamine signaling in the PFC can either accentuate or ameliorate the negative effects of HIV on dopaminergic function. It is proposed that the effect of HIV on working memory is not robust enough to be detected clinically in asymptomatic individuals who have the advantage of the Met allele and the associated increase in prefrontal dopamine levels. In contrast, when considering the inverted, U-shaped dopamine/executive function curve, the combination of the Val/Val and HIV infection appears to push dopamine levels far enough to the left of the curve that executive function and prefrontal function becomes compromised and deficits are observable. Findings reflect the overlap in the adverse effects of HIV and the Val/Val genotype on prefrontal dopaminergic function and suggest that the combination of HIV and the Val/Val genotype surpasses a threshold level of hypodopaminergic

signaling that leads to the manifestation of the effects of HIV on working memory and prefrontal dysfunction. Overall, findings highlight dopamine dysfunction as a neural mechanism underlying HAND and suggest that the decrease in dopamine signaling that results from the combination of HIV and the Val/Val genotype contributes to a vulnerability to working memory impairment and prefrontal processing inefficiency.

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Table 1

Behavioral study: Demographics for participants as a function of HIV serostatus (HIV+, HIV-) and the interaction of HIV serostatus and *COMT* Val158Met genotype group (Val/Val, Met allele carriers).

Background Characteristics	HIV Serostatus (N = 97)		*COMT* Genotype (N = 97)			
			Met allele carriers (n=55)		Val/Val (n = 42)	
	HIV+ (n = 67) M (SD)	HIV- (n = 30) M (SD)	HIV+ (n = 44) M (SD)	HIV- (n = 11) M (SD)	HIV+ (n = 23) M (SD)	HIV- (n = 19) M (SD)
Age at Visit [S]	43.2 (9.7)	35.6 (9.5)	42.6 (9.7)	33.6 (8.5)	44.4 (9.9)	36.7 (10.0)
Graduated high school [SG]	67%	83%	58%	91%	86%	79%
WRAT Reading Test	84.9 (2.2)	92.1 (3.3)	83.8 (19.4)	92.1 (15.4)	85.9 (17.2)	92.2 (13.4)
Depressive Symptoms, CES-D >16	43%	24%	45.2%	18.2%	36.8%	27.8%
Race/Ethnicity						
African-American (non-Hispanic)	82%	80%	82%	91%	83%	74%
White (non-Hispanic)	6%	0%	7%	0%	4%	0%
Hispanic	9%	20%	7%	9%	13%	26%
Other	3%	0%	4%	0%	0%	0%
Hepatitis C Virus Antibody [S]	36%	10%	33%	9%	41%	11%
Recently Used Crack/Cocaine/Heroin	0%	0%	0%	0%	0%	0%
Ever Used Crack/Cocaine/Heroin [S]	64%	40%	64%	45%	65%	37%
Currently Smoking	45%	40%	48%	54%	39%	32%
Hazardous Alcohol Use [SG]	4%	13%	4%	36%	4%	0%
Recently Used Marijuana [G, SG]	9%	23%	13%	45%	0%	10%
Menopause Stage [SG]						
Premenopausal	43%	65%	34%	80%	60%	54%
Perimenopausal	24%	18%	27%	10%	18%	23%

Postmenopausal	33%	17%	39%	10%	23%	23%

Note. [S] Main effect of serostatus significant at $p < 0.05$; [G] Main effect of genotype significant at $p < 0.05$, [SG] Serostatus x Genotype interaction significant at $p < 0.05$; WRAT =Wide Range Achievement Test; CES-D = depressive symptoms measured by Center for Epidemiologic Studies Depression Scale with >16 cut-off; "Recent" refers to within 6 months of the most recent WIHS visit. Hazardous alcohol use reflects >7 drinks per week or more than 4 drinks in one sitting.

Table 2

Behavioral study: Clinical characteristics of HIV+ participants overall and as a function of *COMT* Val158Met genotype group (Val/Val, Met allele)

Clinical Characteristics	HIV+ n = 67 M (SD)	*COMT* Genotype (N = 67) HIV+ Met allele n = 44 M (SD)	HIV+ Val/Val n = 23 M (SD)
CD4 Level			
> 500	45%	52%	32%
> 200 and ≤ 500	42%	34%	56%
< 200	13%	14%	13%
CD4 nadir	336 (165)	357 (172)	292 (143)
Viral Load			
Undetectable	58%	60%	56%
< 10,000	24%	25%	23%
≥ 10,000	17%	14%	22%
Medication Use			
cART	70%	68%	74%
Non-cART	0%	0%	0%
ARV naïve	30%	32%	26%
Medication compliance (> 95%)	83%	77%	94%

Note. CD4 Level = T-Helper cell count per mm^3 of blood; CD4 nadir = lowest recorded CD4 level to date; cART =combined antiretroviral therapy; ART = antiretroviral therapy

Table 3

Imaging study: Demographics for participants as a function of HIV serostatus (HIV+, HIV-) and the interaction of HIV serostatus and *COMT* Val158Met genotype group (Val/Val, Met allele carriers).

	HIV Serostatus		*COMT* Genotype (N = 33)			
			Met allele carriers (n=18)		Val/Val (n = 15)	
Background Characteristics	HIV+ (n = 20) M (SD)	HIV- (n = 13) M (SD)	HIV+ (n = 13) M (SD)	HIV- (n = 5) M (SD)	HIV+ (n = 7) M (SD)	HIV- (n = 8) M (SD)
Age at Visit	43.1 (7.4)	42.7 (10.1)	43.5 (8.0)	44.6 (13.4)	42.4 (6.7)	41.5 (8.2)
Graduated high school	80%	77%	85%	100%	71%	63%
WRAT Reading Test	86.3 (17.6)	84.1 (13.0)	85.8 (4.5)	87.0 (7.3)	87.3 (6.7)	82.2 (5.8)
Depressive Symptoms, CES-D >16	15%	15%	15%	20%	14%	13%
Race/Ethnicity						
African-American (non-Hispanic)	95%	92%	92%	80%	100%	100%
White (non-Hispanic)	5%	8%	8	20	0	0
Hispanic	0%	0%	0	0	0	0
Other	0%	0%	0	0	0	0
Hepatitis C Virus Antibody	21%	15%	33%	9%	41%	11%
Recently Used Crack/Cocaine/Heroin	0%	0%	0%	0%	0%	0%
Ever Used Crack/Cocaine/Heroin	50%	54%	61%	40%	29%	62%
Currently Smoking	50%	31%	54%	80%	43%	63%
Hazardous Alcohol Use	15%	0%	23%	0%	0%	0%
Recently Used Marijuana [T]	10%	20%	15%	40%	0%	10%
Menopause Stage						
Premenopausal	65%	62%	61%	40%	72%	76%
Perimenopausal	10%	15%	8%	40%	14%	0%

Postmenopausal	25%	15%	31%	20%	14%	12%
Unknown	0%	8%	0%	0%	0%	12%

Note. [T] A trend for a significant effect of genotype at $p < 0.1$; WRAT =Wide Range Achievement Test; CES-D = depressive symptoms measured by Center for Epidemiologic Studies Depression Scale with >16 cut-off; "Recent" refers to within 6 months of the most recent WIHS visit. Hazardous alcohol use reflects >7 drinks per week or more than 4 drinks in one sitting.

Table 4

Imaging study: Clinical characteristics of HIV+ participants overall and as a function of *COMT* Val158Met genotype group (Val/Val, Met allele)

Clinical Characteristics	HIV+ n = 20 M (SD)	*COMT* Genotype (N = 20) HIV+ Met allele n = 13 M (SD)	HIV+ Val/Val n = 7 M (SD)
CD4 Level			
> 500	59%	54%	67%
> 200 and ≤ 500	35%	46%	17%
< 200	6%	0%	16%
CD4 nadir	336 (165)	317 (151)	287 (127)
Viral Load			
Undetectable	60%	61%	57%
< 10,000	25%	23%	29%
≥ 10,000	15%	15%	14%
Medication Use			
cART	85%	85%	85%
Non-cART	0%	0%	0%
ARV naïve	11%	15%	0%
Medication compliance (> 95%)	82%	82%	83%

Note. CD4 Level = T-Helper cell count per mm^3 of blood; CD4 nadir = lowest recorded CD4 level to date; cART =combined antiretroviral therapy; ART = antiretroviral therapy

Table 5

fMRI study: Brain region, Brodmann area (BA), Talairach coordinates, cluster size (k) and statistical information for clusters that show significantly greater activation in the 2-back versus the 1-back.

Brain Region	BA	Talairach Coordinates (x, y, z)	Cluster size (k)	Z score	P-value (uncorrected)
L Posterior Parietal Cortex	19	-31, -67, 41	315	4.04	$p < 0.001$
	39	-37, -61, 39	137	3.86	$p < 0.001$
R Posterior Parietal Cortex	19	32, -69, 38	276	3.57	$p < 0.001$
	7	6, -72, 48	41	3.24	$p = 0.001$
R Dorsolateral PFC	9	36, 8, 38	66	3.66	$p < 0.001$
L Dorsolateral PFC	9	-44, 13, 28	23	3.49	$p < 0.001$
R Superior Frontal Gyrus	8	2, 15, 49	423	3.11	$p = 0.001$
L Anterior Cingulate	32	-5, 16, 51	49	3.60	$p < 0.001$
L Inferior Frontal Gyrus	44	-42, -2, 29	15	3.04	$p = 0.001$

Table 6

fMRI study: Brain region, Brodmann area (BA), Talairach coordinates, cluster size (k) and statistical information for significantly active brain regions associated with the serostatus by genotype interaction and the follow-up contrasts

Brain Region	BA	Talairach Coordinates (x, y, z)	Cluster size (k)	Z score	P-value (uncorrected)	Contrast
L Anterior Cingulate	24	-8, 26, 5	66	3.36	$p < 0.001$	Val/Val: HIV+ > HIV- Met carriers: HIV- > HIV+
R DLPFC	10	20, 48, -2	50	3.19	$p = 0.001$	Val/Val: HIV+ > HIV-
R Medial Frontal Gyrus	11	23, 41, 3	29	3.07	$p = 0.001$	Met carriers: HIV- > HIV+
	10	5, 46, 12	16	2.73	$p = 0.003$	Val/Val: HIV+ > HIV-
R Anterior Cingulate	32	8, 30, 22	37	3.08	$p = 0.001$	Val/Val: HIV+>HIV-
		16, 39, 14	32	2.81	$p = 0.003$	Val/Val: HIV+>HIV-
	25	2, 2, -3	26	2.87	$p = 0.002$	Val/Val: HIV+>HIV-

Table 7

fMRI study: Brain region, Talairach coordinates, cluster size (k), and statistical information for significantly active brain regions associated with the serostatus by genotype by N-back condition interaction and the follow-up contrasts

Brain Region, Brodmann Area	BA	Coordinates (x, y, z)	Cluster size (k)	Z score	P-value (uncorrected)	Contrast
R Anterior Cingulate	32	1, 30, 29	174	4.12	$p < 0.001$	Val/Val: HIV+>HIV- on 2back
L Medial Frontal Gyrus	9	-11, 21, 39	15	3.35	$p < 0.001$	Val/Val: HIV+>HIV- on 2back

Figure Captions

Figure 1. The theoretical U-shaped curve relationship between prefrontal cortex dopamine levels and executive function performance and the level of prefrontal dopamine for Val/Val and Met/Met genotypes in individuals with schizophrenia.

Figure 2. Pictorial depiction of the 1-back and 2-back conditions of the N-back test

Figure 3. Behavioral study: Adjusted mean N-back percent accuracy (per trial and average total) as a function of serostatus for Met carriers (left panel) and Val/Val carriers (right panel). * Significant main effect of serostatus groups across N-back conditions, $p < 0.05$; ∓ Significant main effect of N-back condition, $p < 0.05$; **Significant serostatus by genotype interaction, $p < 0.01$.

Figure 4. Brain activation observed in HIV-uninfected controls across all conditions of the N-back test

Figure 5. Brain activation that is greater in HIV-infected compared to HIV-uninfected women in Val/Val carriers (left panel) and Met allele carriers (right panel)

Figure 6. Brain activation that is greater in HIV-uninfected compared to HIV-infected women in Val/Val carriers (left panel) and Met allele carriers (right panel)

Figure 1

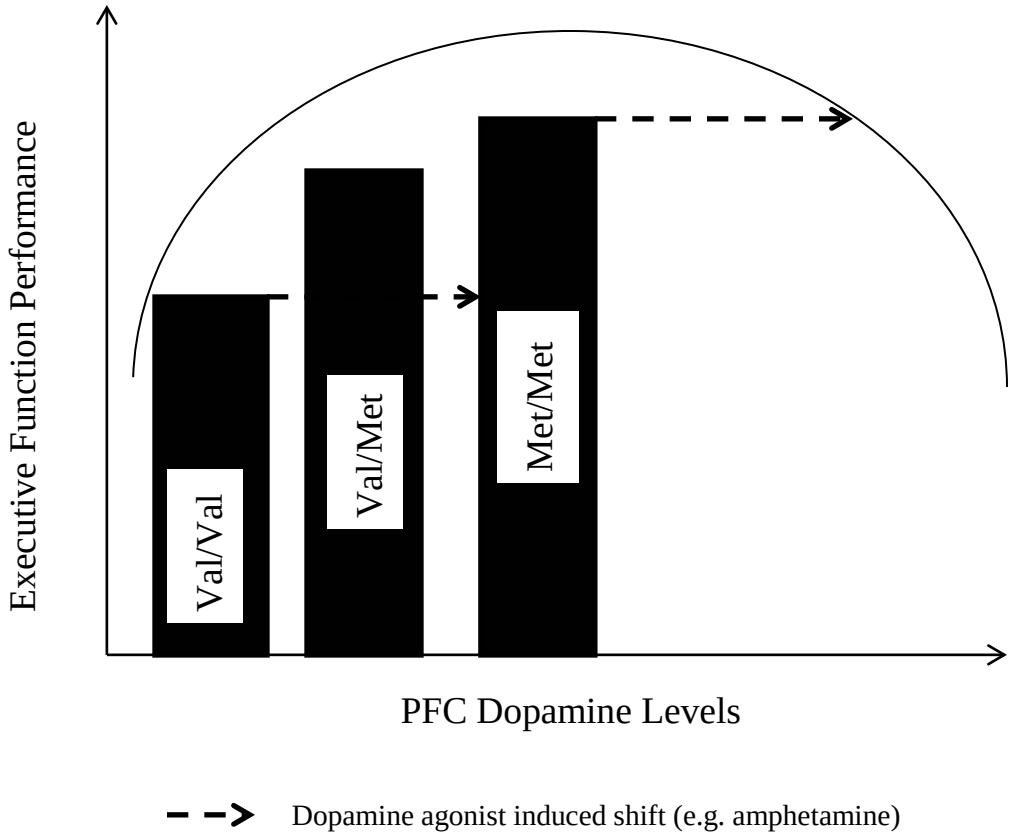
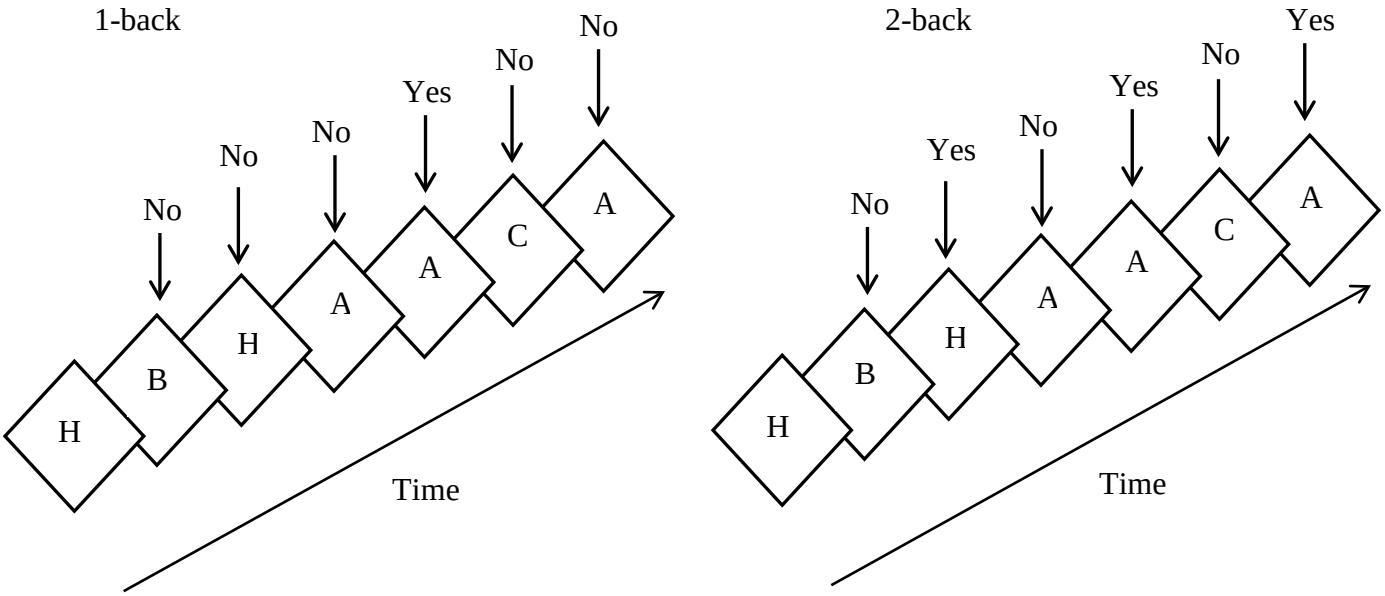

Executive Function Performance
Val/Val
Val/Met
Met/Met
PFC Dopamine Levels
Dopamine agonist induced shift (e.g. amphetamine)

Figure 2


1-back
No
No
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Yes
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2-back
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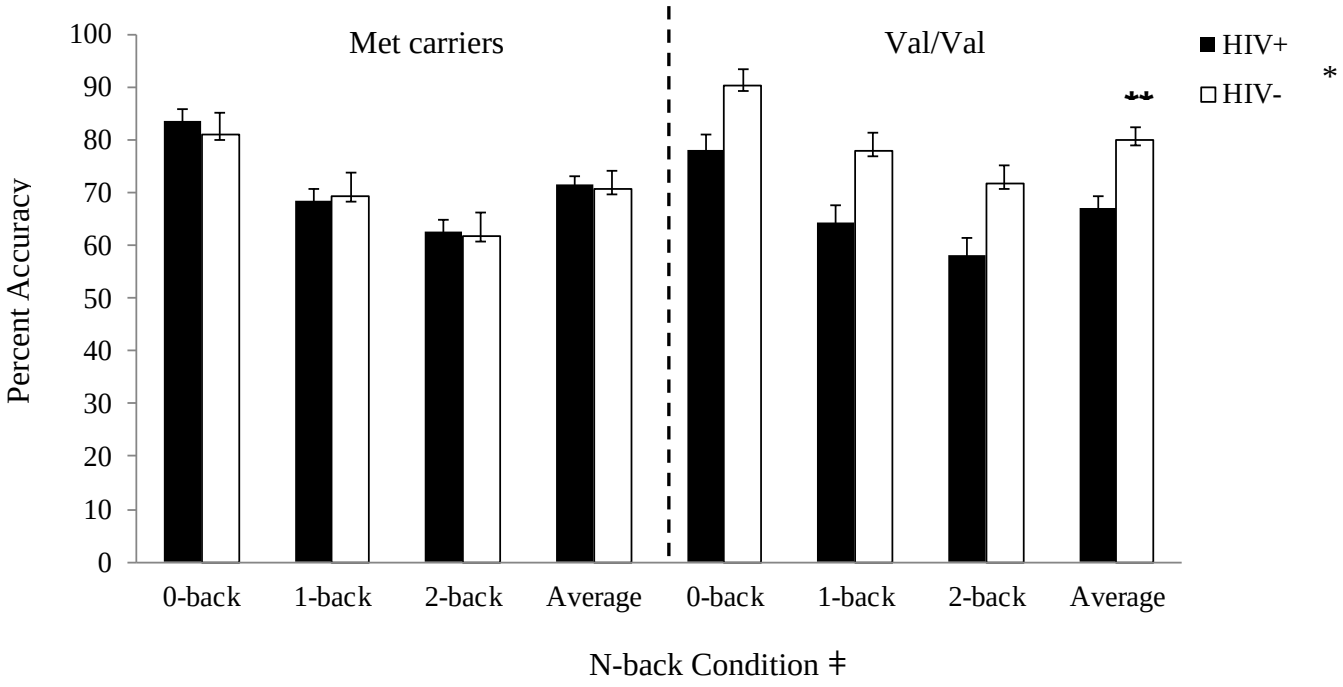
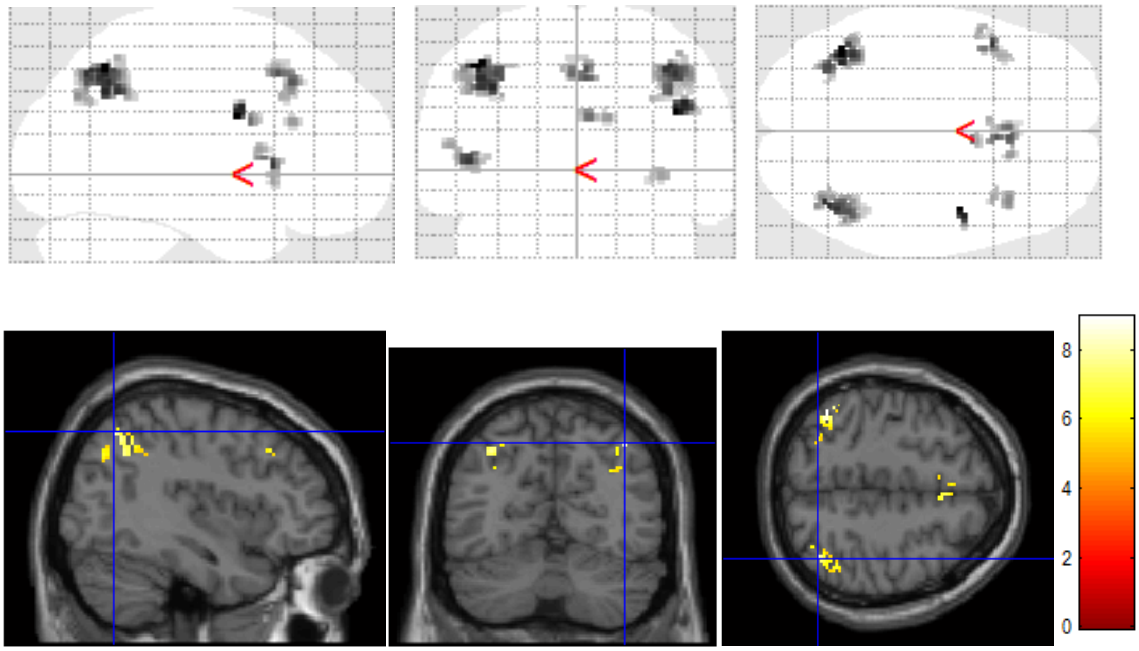
Time

Figure 3



Note. * Significant main effect of serostatus groups across N-back conditions, $p < 0.05$; ǂ Significant main effect of N-back condition, $p < 0.05$; **Significant serostatus by genotype interaction, $p < 0.01$

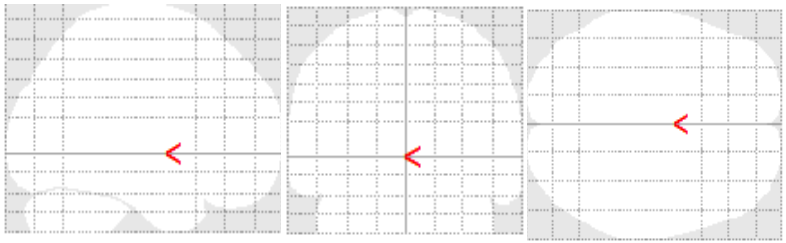
Figure 4



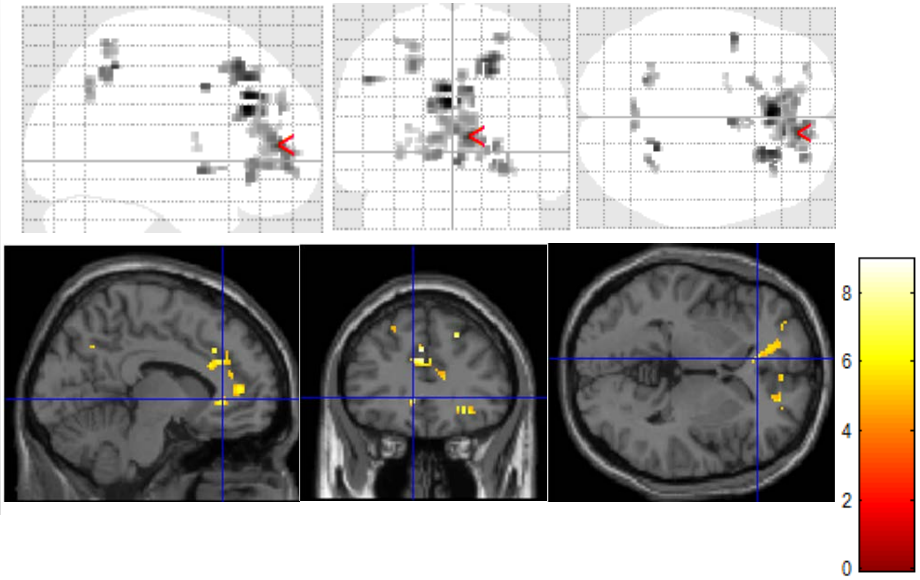
Note. $p < 0.01$

Figure 5

Met carriers: HIV+ > HIV-

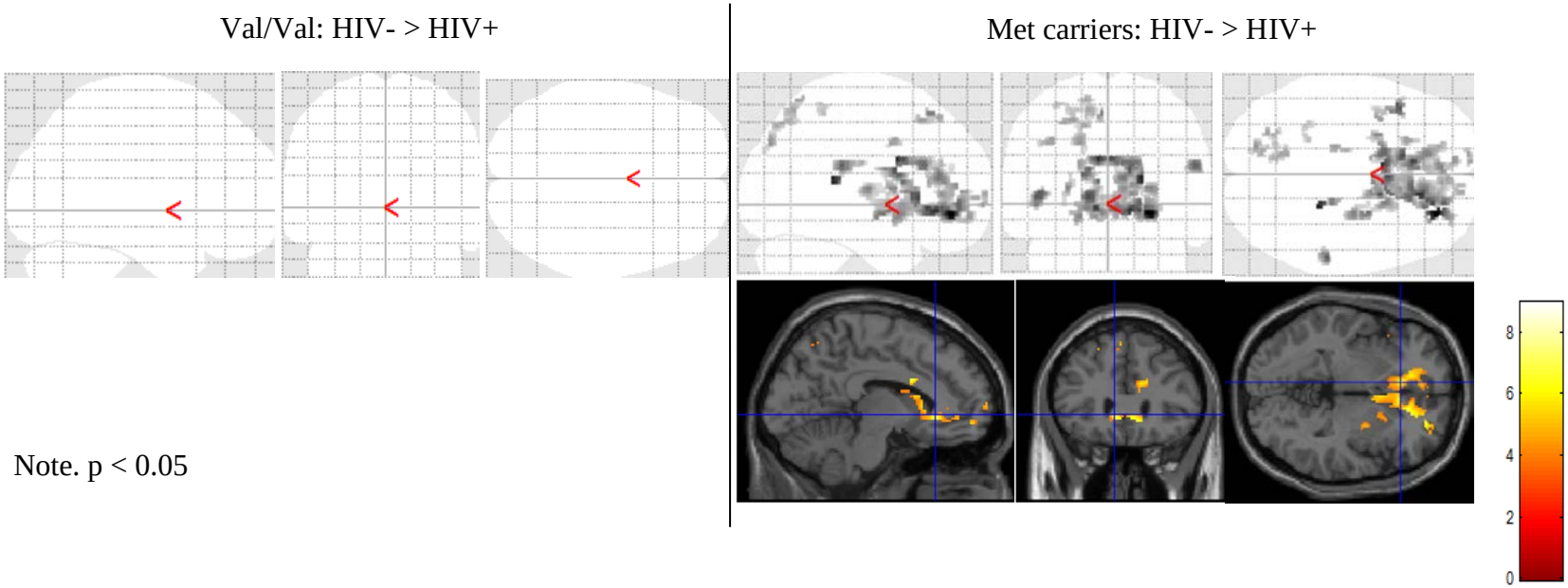


Val/Val: HIV+ > HIV-



Note. $p < 0.05$

Figure 6



Note. $p < 0.05$

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

March 12, 2012

Pauline M. Maki, PhD
Psychiatry
912 S. Wood Street, Room 120G
M/C 913
Chicago, IL 60612-7325
Phone: (312) 996-6941 / Fax: (312) 413-7856

RE: Protocol # 2008-0519
"Genetic Predictors of Cognition in HIV+ Women"

Dear Dr. Maki:

Please note that ***Kathleen Weber's*** research training expired on **10/18/2011** and she must complete a minimum of two hours of continuing education in order to participate in the conduct of the research. You may refer her to the OPRS website, where continuing education offerings are available: http://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/education/2-2-2/ce_requirements.shtml

Your Continuing Review was reviewed and approved by Members of IRB #1 by the Expedited review process on March 7, 2012. You may now continue your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: March 7, 2012 - March 6, 2013
Approved Subject Enrollment #: 0 at UIC; 142 at Chicago WHIS CORE Facility (Enrollment closed)
Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC, Women's Interagency HIV Study, Chicago cohort
Sponsor: None
Research Protocol(s):

a) Multicenter AIDS Cohort Study / Women's Interagency HIV Study: Genetic Predictors of Working Memory and Prefrontal Cortext Dysfunction in HIV+ Women; Version #2, 05/13/2009

Informed Consent(s): N/A; Enrollment Closed.
HIPAA Authorization(s): N/A; Enrollment Closed.

Your research continues to meet the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific category:

(7) Research on individual or group characteristics or behavior (including but not limited to research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
02/28/2012	Continuing Review	Expedited	03/07/2012	Approved

Please remember to:

→ Use your **research protocol number** (2008-0519) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"**UIC Investigator Responsibilities, Protection of Human Research Subjects**"

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 355-2939. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Jewell Hamilton, MSW
IRB Coordinator, IRB # 1
Office for the Protection of Research Subjects

Enclosure(s):

1. **UIC Investigator Responsibilities, Protection of Human Research Subjects**

cc: Anand Kumar, Psychiatry, M/C 912

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Continuing Review

August 24, 2011

Pauline M. Maki, PhD
Psychiatry
912 S. Wood Street, Room 120G
M/C 913
Chicago, IL 60612-7325
Phone: (312) 996-6941 / Fax: (312) 413-7856

RE: Protocol # 2009-0652
"Predictors of Brain Functioning in Women with HIV"

Dear Dr. Maki:

Your Continuing Review was reviewed and approved by the Expedited review process on August 16, 2011. You may now continue your research.

Please note the following information about your approved research protocol:

Please note that the required Appendix M was not included with the Continuing Review submission. Please submit a completed Appendix M as an amendment to the IRB within 90 days.

Protocol Approval Period: August 16, 2011 - August 14, 2012
Approved Subject Enrollment #: 78 (57 enrolled to date)
Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC, Cook County CORE Center
Sponsor: 1) NIAID, NIDA, NCI, 2) National Institute on Drug Abuse (NIDA/NIH)
PAF#: 1) 2010-00282, 2) 2009-06134
Grant/Contract No: 1) 2 U01AI034993 (NIAID), 2) F31DA028573
Grant/Contract Title: 1) Effects of Drug Use on Hippocampal Function in HIV+ Women, 2) Effects of Drug Use on Prefrontal Cortex Function in HIV+ Women
Research Protocol(s):

b) Predictors of Brain Functioning in Women with HIV, Version #7, 05/04/2011

Recruitment Material(s):

a) White Matter Integrity in HIV+ women, Information Sheet, Version 2.0, 11/02/10
b) White Matter Integrity in HIV+ women, UIC Telephone Script, Version 3.0, 11/30/10

c) White Matter Integrity in HIV+ women, PBF Screening Script, Version 3.0, 12/15/10

Informed Consent(s):

a) Predictors of Brain Function in HIV+ Women, Version 7; 05/04/2011

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific categories:

(4) Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving X-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.).,

(5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis).,

(7) Research on individual or group characteristics or behavior (including but not limited to research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

Please note the Review History of this submission:

Receipt Date	Submission Type	Review Process	Review Date	Review Action
08/11/2011	Continuing Review	Expedited	08/16/2011	Approved

Please remember to:

→ Use your **research protocol number** (2009-0652) on any documents or correspondence with the IRB concerning your research protocol.

→ Review and comply with all requirements on the enclosure,
"**UIC Investigator Responsibilities, Protection of Human Research Subjects**"

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 413-7323. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Jennifer Joaquin, MPH
IRB Coordinator, IRB # 1
Office for the Protection of Research Subjects

Enclosure(s):

2. **UIC Investigator Responsibilities, Protection of Human Research Subjects**
3. **Informed Consent Document(s):**
   a) Predictors of Brain Function in HIV+ Women, Version 7; 05/04/2011
4. **Recruiting Material(s):**
   a) White Matter Integrity in HIV+ women, Information Sheet, Version 2.0, 11/02/10
   b) White Matter Integrity in HIV+ women, UIC Telephone Script, Version 3.0, 11/30/10
   c) White Matter Integrity in HIV+ women, PBF Screening Script, Version 3.0, 12/15/10
5. **Data Security Enclosure**

cc: Anand Kumar, Psychiatry, M/C 912
OVCR Administration, M/C 672

Curriculum Vitae

Name: Erin Sundermann
Address: Department of Psychiatry
Neuropsychiatric Institute
912 South Wood Street, MC 913
Chicago IL. 601612
Phone: (312)996-9029
E-mail: esundermann@psych.uic.edu

Education:

- Pursuing a Ph.D. degree in Psychology, University of Illinois, Chicago
  - Current GPA – 3.8
- MA Psychology, San Diego State University, San Diego, California, 2005
  - Overall GPA – 4.0
- BS Behavioral Neuroscience, Lafayette College, Easton, Pennsylvania, 2001
  - Overall GPA – 3.6 Junior/Senior GPA – 3.8
  - Major GPA – Behavioral Neuroscience 3.6

Honors and Awards:

- **Alice Dan Dissertation Research Award** ($500) from the Center for Research on Women and Gender (2011)
- **$1,000 Scholarship** to attend the 52st Annual Short Course on Medical and Experimental Mammalian Genetics at the Jackson Laboratory in Bar Harbor, Maine (2011)
- Top five winner of the **Under 34 Competition of the 14th World Congress of Gynecological Endocrinology** (2010)
- **Young Investigator Travel Award from the North American Menopause Society** for attendance at the 2009 annual meeting (2009)
- **Student liaison to the Academic Program Review Committee** for the MA Psychology program at San Diego State University (2005)
- **Student Travel Award** from the Association for Chemoreception Sciences 27th annual meeting (2005)
- **Graduated Cum Laude**, Lafayette College, Easton, PA. (2001)
- **Psychology Honor Society** - Psi Chi (2000)
- **Dean's List**, Lafayette College (1997-2001)

Research Support:

- **Chancellor's Supplemental Research Fellowship,** University of Illinois at Chicago (2010-2012)
  Project Title: Genetic Predictors of Cognition in HIV+ Women
  Role: PI
  Award: $8,000
- **Scholar's Grant from Mt. Sinai Institute of NeuroAIDS Disparities** (2008)
  Project Title: Genetic Predictors of Prefrontal Function in HIV+ Women

Role: PI
Award: $25,000 and attendance at a six week Summer Institute aimed to support research in NeuroAIDS disparities

- **F31 Ruth L. Kirschstein National Research Service Award**, NIMH (2008-2011)
  Project Title: Genetic Predictors of Cognition in HIV+ Women
  Role: PI
  Award: $32,972 (annual direct)

Publications:

Sundermann, E., Maki, P. & Bishop, J. (2010). A Review of Estrogen Receptor á Gene (ESR1) Polymorphisms, Mood, and Cognition. *Menopause*, 17(4), 874-886.

Maki, P. & **Sundermann, E**. (2009). Hormone therapy and cognitive function. *Human Reproduction*, 15(6), 667-81.

Sundermann, E., Gilbert, P.E., & Murphy, C. (2008) The effect of hormone therapy on olfactory sensitivity is dependent on apolipoprotein E genotype. *Hormones and Behavior*, 54(4), 528-33.

Sundermann, E., Gilbert, P.E. & Murphy, C. (2007) Apolipoprotein E ε4 genotype and gender: effects on memory. *The American Journal of Geriatric Psychiatry*, 15(10), 869-878.

Sundermann, E., Gilbert, P.E., & Murphy, C. (2006) The effect of estrogen on recognition memory for visual and olfactory stimuli in females diagnosed with Alzheimer's disease, *Journal of the International Neuropsychological Association*, 12, 400-404.

Manuscripts in Preparation:

Sundermann, E., Bishop, J.R., Rubin, L.H., Martin, E., Kreek, M.J., Levran, O., Randesi, M., Weber, K., Cohen, M. & Maki, P.M. Impact of catechol-O-methyltransferase Val158Met (rs4680) genotype on verbal working memory in women with HIV

Sundermann, E., Rubin, L., Bishop, JR., Weber, K., Cohen, M., Valcour, V., Crystal, H., Golub, E., Karim, R., Anastos, K., Liu, C., Maki, P.M. *CYP1A1* Polymorphisms Predict Verbal Memory in Ethnically Diverse, Low-Income Women with Prevalent HIV.

Conference Presentations:

Grauzas, V., **Sundermann, E**., Little, D., Weber, K., Cohen, M., & Maki, P. (October, 2012). Alterations in hippocampal functioning in HIV-infected women. 2012 Annual Meeting of the Society for Neuroscience, New Orleans, LA.

Sundermann, E., Bishop, J.R., Rubin, L., Weber, K., Cohen, M., Manly, J., Martin, E., & Little, D. (September, 2011). Impact of catechol-O-methyltransferase Val158Met (rs4680) genotype on verbal working memory in women with HIV. The XIXth Annual Meeting of the World Congress of Psychiatric Genetics, Washington D.C.

Sundermann, E., Rubin, L.H., Bishop, J.R., Weber, K., Cohen, M., Crystal, H., Valcour, V., Golub, E.T., Karim, R., Anastos, K., Liu, C., Maki, P.M. (November 2010) The impact of cytochrome P450 polymorphisms on verbal memory in HIV infected and at risk women. The Annual Meeting of the American Society of Human Genetics, Washington D.C.

Sundermann, E., Rubin, L.H., Bishop, J.R., Weber, K., Cohen, M., Golub, E., Anastos, K., Crystal, H., Chenglong, L., Aouizerat, B., Pearce, C., Maki, P.M. (October, 2010) The impact of estrogen-related polymorphisms on risk of menopause symptoms in a large cohort of ethnically diverse, low income women. 21st Annual Meeting of the North American Menopause Society, Chicago, IL.

Sundermann, E., Rubin, L. H., Martin, E. M., Weber, K., Cohen, M., Crystal, H. Golub, E. T., Greenblatt, R. M. & Maki, P. M. (September, 2009). The Impact of Menopausal Phase and Menopausal Symptoms on Cognitive Dysfunction in Women with HIV. North American Menopause Society. San Diego, CA.

Rubin, L.H., **Sundermann, E**., Cohen, M., Golub, E.T., Greenblatt, R., Young, M. Schwartz, R., Anastos, K., Cook, J.A., Maki, P.M. (September, 2009). The influence of menopausal status on depressive symptoms in the Women's Interagency HIV Study. North American Menopause Society 20th Annual Meeting. San Diego CA.

Sundermann, E., Levran, O., Robinson-Papp, J., Bishop, JR. & Morgello, S. (July, 2009) Association between the Catechol-O-Methyltransferase (COMT) gene variant, Val158Met, and motor deficits in HIV+ patients. Annual Meeting of the International Society of Neurovirology. Miami, Florida.

Sundermann, E., Rubin, L., Maki, P., Martin, E., Cohen, M., Urwin, S., Weber, K. (May, 2009). Perceived Stress is Higher in HIV+ Women and Negatively Impacts Cognitive Function. Society for Biological Psychiatry. Vancouver, Canada.

Sundermann, E., Rubin, L.H., Mordecai, K.L., Eatough, E., & Maki, P.M. (February 2009) The effects of stress and oral contraceptives on cognitive flexibility. Annual Meeting of the International Neuropsychological Society. Atlanta GA.

Sundermann, E., Martin, E., Rubin, L., Gould, F., Cohen, M., Weber, K., & Maki P. (May, 2008) Executive/frontal dysfunction contributes to verbal memory impairment in HIV+ women. Annual Meeting of the Society for Biological Psychiatry, Washington D.C.

Sundermann, E., Martin, E., Rubin, L., Gould, F., Cohen, M., Weber, K., & Maki P. (February, 2008) Executive/frontal dysfunction contributes to verbal memory impairment in HIV+ women. University of Illinois, Chicago, College of Medicine Research Symposium, Chicago, IL.

Sundermann, E., Rubin, L., Mordecai, K., & Maki, P. (February, 2008). Relationships between nonverbal and verbal measures of fluency. 36th Annual Meeting of the International Neuropsychological Society, Kona, HI.

Sundermann, E., Rubin, L., Martin, E., Cohen, M., Weber, K., & Maki, P. (August, 2007). Relationships among menopausal symptoms, sex steroid hormones and cognitive dysfunction in women with HIV. Annual Meeting of the International Society of Psychoneuroendocrinologists, Madison, WI.

Sundermann, E., & Murphy, C. (February, 2006). The effect of Gender and the apolipoprotein E ε4 allele on rate of decline in recognition memory for olfactory stimuli in patients diagnosed with Alzheimer's disease. Annual Meeting of the International Neuropsychological Society, Boston, MA.

Sundermann, E., Gilbert, P.E., & Murphy, C. (April, 2005) The effect of estrogen and its interaction with the ApoE epsilon 4 genotype on olfactory functioning in nondemented elderly females and females diagnosed with Alzheimer's disease. Association for the Chemoreception Sciences Annual Meeting, Sarasota, FL.

Gilbert, P.E., **Sundermann, E.,** & Murphy, C. (April, 2005). The effect of gender and the apolipoprotien E ε4 allele on recognition memory for olfactory and visual stimuli in patients diagnosed with Alzheimer's disease and healthy older adults. Association for the Chemoreception Sciences Annual Meeting, Sarasota, FL.

Sundermann, E., Gilbert, P.E., & Murphy, C. (February, 2005). The effect of estrogen on recognition memory for visual and olfactory stimuli in females diagnosed with Alzheimer's disease. Annual Meeting of the International Neuropsychological Society, St. Louis, MO.

Murphy, C., Gilbert, P.E. & **Sundermann, E**. (October, 2004). Excellent sensitivity and specificity for Alzheimer's disease in a brief test of dementia. Society for Neuroscience Abstracts, San Diego, CA.

Invited Talks:

International

Sundermann, E. (March, 2010). The Effects of Menstrual Cycle Phase and Stress on Cognitive Flexibility. Annual Meeting of the International Society of Gynecological Endocrinology. Florence, Italy.

National

Sundermann, E. (October, 2010). HIV, Menopause, and Women's Mental and Cognitive Health. Centers for AIDS Research (CFAR) Joint Symposium on HIV research in Women. Chicago, IL.

Sundermann, E. (July, 2009). Genetic Predictors of Cognition in HIV+ Women. Alumnae Symposium of the Mt. Sinai Institute for NeuroAIDS Disparities. New York, NY.

Local

Sundermann, E. (February, 2012).Genetic Predictors of Cognition and Prefrontal Function in Women with HIV. Monthly Meeting of the Institute for Human Genetics, University of Illinois at Chicago, Chicago, Illinois.

Sundermann, E. (April, 2010).Genetic Predictors of Cognition in HIV+ Women. Behavioral Neuroscience Brownbag. University of Illinois at Chicago, Chicago, Illinois.

Sundermann, E. (March, 2010). Genetic Predictors of Cognition in HIV+ Women. fMRI Journal Club in the Center for Cognitive Medicine. University of Illinois at Chicago. Chicago, Illinois.

Sundermann, E. (October, 2009). WIHS-IV Neurocognitive Progress Report. Chicago WIHS Consortium Meeting, Chicago, Illinois.

Sundermann, E. (March, 2009). Executive/Frontal Dysfunction Contributes to Verbal Memory Impairment in HIV+ Women. Behavioral Neuroscience Brownbag. University of Illinois at Chicago, Chicago, Illinois.

Sundermann, E. (April, 2008). The Effects of Stress and Oral Contraceptives on Cognitive Flexibility. Behavioral Neuroscience Brownbag. University of Illinois at Chicago, Chicago, Illinois.

Sundermann, E. (April, 2007). Menopause and Cognitive Dysfunction in Women with HIV. Behavioral Neuroscience Brownbag. University of Illinois at Chicago, Chicago, Illinois.

Service to the Profession:

Articles Reviewed

- Psychoneuroendocrinology
- Neurological Sciences
- Journal of Gerontology
- Journal of Affective Disorders

Articles Co-Reviewed

- Psychoneuroendocrinology
- Neurology
- Psycho-oncology
- Brain and Cognition
- Maturitas

Research Experience:

- Graduate Research Assistant, Department of Psychiatry, Center for Cognitive Medicine, University of Illinois at Chicago. Chicago, IL (2006-present)
- Neurocognitive testing consultant for the Women's Interagency HIV Study (2008-present)
- Research intern in the Laboratory of the Biology of Addictive Diseases, Rockefeller University, New York, NY. (2008)
- Graduate Research Assistant, Department of Psychology, Life Span Human Senses Laboratory, San Diego State University. San Diego, CA (2003-2006)

- Staff Research Associate, Department of Medical Genetics, University of California San Diego. San Diego, CA. (2001-2006)
- Research intern at the Children's Hospital Medical Genetics Laboratory. Cincinnati, Ohio (2000)
- Research intern at the Sainsbury Center Research Department. London, England (1999)

Teaching Experience

- Graduate Teaching Assistant, Introduction to Psychology. Department of Psychology, University of Illinois at Chicago. Chicago, IL (2007-2008)
- Graduate Teaching Assistant, Physiological Psychology. Department of Psychology, University of Illinois at Chicago. Chicago, IL (2008)
- Teacher's Assistant, Molecular Biology, Department of Biology, Lafayette College. Easton, PA. (2001)

Functional Magnetic Resonance Imaging (fMRI) Experience

- Participated in a fMRI Image Acquisition and Analyses using SPM offered by the Mind Research Institute at the University of New Mexico (2010)
- Certified as an operator of 3T GE scanners (2005 – 2008)
- Participated in a fMRI and AFNI processing workshop at the Medical College of Wisconsin (2004)