Facultad de Psicología

DOCTORAL THESIS

Neuropsychological pattern of aviremic patients infected by human immunodeficiency virus and effect of treatment with protease inhibitor monotherapy

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UNIVERSIDAD AUTÓNOMA DE MADRID

2015

A mis abuelos

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Neuropsychological pattern of aviremic patients infected by human immunodeficiency virus and effect of treatment with protease inhibitor monotherapy

Perfil neuropsicológico del paciente infectado por el virus de la inmunodeficiencia humana y efecto del uso de inhibidores de la proteasa en monoterapia

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Programa de doctorado en Psicología Clínica y de la Salud.

Neurociencia de la cognición y la emoción (2011-2015)

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Octubre de 2015

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Agradecimientos (Acknowledgments)

En primer lugar quisiera agradecer a todos los participantes que desinteresadamente ceden su tiempo y permiten el desarrollo de investigaciones destinadas a maximizar la calidad de vida de las personas infectadas por VIH. Gracias de manera especial a los pacientes de la Unidad de VIH del Hospital La Paz porque ellos me han enseñado lo que los libros no explican y gracias a ellos he mantenido la motivación en los momentos más arduos del proceso.

Gracias a las personas fundamentales de este proyecto. A Carmen Bayón por enseñarme a hacer entrevistas clínicas y por las horas de supervisión. A Irene Cambrón por ayudarme a poner en marcha las evaluaciones neuropsicológicas. A Susana por hacer con sus explicaciones que los análisis de datos fueran más fáciles e interesantes. A Miriam por todas las horas dedicadas. A Nacho P., por creer en la neuropsicología como complemento de trabajo, por las horas de debates y sobre todo por su paciencia. A todos los co-autores de los trabajos publicados por su implicación y consejos.

También mi agradecimiento a los médicos, auxiliares de enfermería y enfermeros del Hospital La Paz y Doce de Octubre de Madrid, que han colaborado en el reclutamiento y la buena marcha del proyecto. A Gema José por haber participado en las evaluaciones neuropsicológicas. A mis compañeros de la Unidad de VIH del Hospital La Paz, por haberme enseñado el significado de trabajar en equipo y por ser modelos de calidad profesional y personal. A todos los que han trabajado en el aula en estos años por el tiempo de trabajo compartido. A Marisa, Pancho y Nacho B. por su implicación y apoyo continuo.

Gracias a Ana y Alejandro por dedicarme su tiempo, estar siempre dispuestos a ayudarme y hacer de los meses de rotación en Londres una experiencia realmente interesante.

Gracias a Jose Ramón Arribas por haber aceptado tutorizar a una psicóloga totalmente inexperta en el mundo de la investigación. Por haberme enseñado a pensar de manera científica y por las horas dedicadas a mi escritura.

A Fernando Carvajal por aceptar dirigir esta tesis, por estar siempre disponible y por enseñarme tanto sobre interpretación de evaluaciones. Gracias por tu confianza y pensar en mi ante nuevos proyectos.

Gracias a mis compañeros de carrera, con los que compartí las primeras experiencias relacionadas con la psicología y una de una de las mejores etapas de mi vida. A los psicólogos de Hematooncología que, muy al principio, hicieron que confirmara que quería dedicarme a esta profesión: Alicia Alameda, Javier Barbero, Martina Gulini y Laura Díaz. A Carola del Rincón por su generosidad, por ser modelo de profesional ideal y por confiar en mi desde los primeros momentos. Gracias al resto de psicólogas, ya amigas, con las que he compartido formación de postgrado y con las que siempre puedo contar: Ana, Anama, Gema, Joanna, Marta y Noe.

Gracias también a Carlos, Maikel y Tere por su constante e inigualable soporte y amistad. A toda mi familia, especialmente a mis padres por apoyarme en cada proyecto. A mi madre por siempre estar. A mi padre por sus valores. A mis sobrinos por haber sido la mejor excusa para parar y por regalarme los momentos más auténticos. Y por último, gracias Javi por cuidarme en este proceso; por los cafés, los masajes y los planes aplazados; por los viajes a Londres; por apoyarme en todo; y por hacer mi vida tan fácil.

List of abbreviations/ Lista de abreviaturas

Abbreviations in English

AIDS: Acquired Immunodeficiency Syndrome

ANI: Asymptomatic Neurocognitive Impairment

ANN: American Association of Neurology

ANOVA: Analysis of Variance

ART: Antiretroviral Therapy

BVMT-R: Brief Visuospatial Memory Test - Revised

CD4: CD4+ T-Lymphocytes

CHO: Choline

CNS: Central Nervous System

CPE: Central Nervous System Penetration-Effectiveness

DSM-IV: Diagnostic and Statistical Manual of Mental Health Disorders

FAB: Florida Affect Battery

HAD: HIV-Associated Dementia

HADS: Hospital Anxiety and Depression Scale

HAND: HIV-Associated Neurocognitive Disorders

HCV: Hepatitis C Virus

HDL: High Density Lipoprotein

HIV: Human Immunodeficiency Virus-type 1

HIV+ people: HIV-Infected People

HOMA-IR: Homeostasis Model Assessment of Insulin Resistance

IQ: Intelligence Quotient

IQR: Interquartile Range

MI: Myo-Inositol

MND: Mild Cognitive Disorder

MRI: Magnetic Resonance Imaging

MRS: Magnetic Resonance Spectroscopy

MT: Monotherapy

NAA: N-Acetyl-Aspartate

NCI: Neurocognitive Impairment

PI: Protease Inhibitor

RNA: Ribonucleic Acid

SD: Standard Deviation

TT: Triple Therapy

VSRT: Verbal Selective Reminding Test

WAIS-III: Wechsler Adult Intelligence Scale

Abreviaturas en español

VIH: Virus de Inmunodeficiencia Humana-tipo 1

SNC: Sistema Nervioso Central

TAR: Tratamiento Antiretroviral

Context of the doctoral thesis

The empirical analyses that composed the present doctoral thesis have been carried out in the human immunodeficiency virus Unit of the Hospital Universitario La Paz (Madrid, Spain). Our analyses are nested in the Picasso Study, a multicentre longitudinal study financed by Instituto de Salud Carlos III (PI10/00483). Also supported by Instituto de Salud Carlos III, the doctoral candidate completed a predoctoral fellowship (FI11/00338) (September 2011 to September 2015). In this period she participated in the study implementation, conducted the neuropsychological assessments and data analysis, reported results at conferences, and produced published articles.

The Picasso Study was designed to evaluate the impact on neurocognition of boosted protease inhibitor in monotherapy, an antiretroviral treatment strategy used to limit toxicity related to nucleoside reverse transcriptase inhibitors. The study compared on several markers of the central nervous system (CNS) functioning a group of HIV-infected (HIV+) patients receiving protease inhibitors monotherapy and a control group receiving protease inhibitors as part of triple therapy regimens.

The Picasso Study included a total of 191 HIV+ patients who completed a comprehensive neuropsychological assessment and a blood test at the baseline visit. At the inclusion, all patients were receiving effective antiretroviral therapy (ART) for at least one year. Of the total, 69% (n=134) accepted to repeat the same procedures at one year follow-up visit. Nested in the Picasso Study, the Instituto de Salud Carlos III approved a sub-study in which several volumetric and inflammatory markers were compared by group of treatment (n=39). The inclusion criteria of the neuroimaging sub-study required that half of patients had global neurocognitive impairment (NCI) at baseline.

We conducted three empirical analyses, all sub-studies of the Picasso Study. The first analysis compared HIV+ patients receiving protease inhibitor in monotherapy or as part of triple therapy in several measures of cognitive and motor functioning. This analysis also explored the pattern of cognitive and motor functioning in our whole sample of HIV+ patients, regardless of the type of ART. The analysis included all patients who completed the basal visit of the Picasso Study (Analysis 1).

The second analysis explored the emotion processing of facial expression, for the first time in a sample of long-term aviremic HIV+ patients (Analysis 2). Our third analysis explored the vocal emotional processing and their cerebral correlates, for the first time in a HIV+ sample (Analysis 3). A protocol of emotional processing tasks was conducted as part of the follow-up neuropsychological assessment of the Picasso Study.

Results of the Analysis 1 have been previously published in González-Baeza et al. (2014), and data of the Analyses 2 and 3 are in preparation for publication.

1. BACKGROUND AND SUMMARY/ANTECEDENTES Y RESÚMEN

1.1. Background and summary: English version

Infection by human immunodeficiency virus- type 1 (HIV) remains highly prevalent worldwide and remains a major public health problem (UNAIDS, 2013). Since Luc Montagnier isolated the virus in 1983, cases of dementia associated with progression of HIV infection in the CNS have been reported (Navia, Cho, Petito, & Price, 1986). The lack of effective treatment in the earliest years of the epidemic meant that HIV could invade the CNS and cause neuroinflammation and dementia. Since the introduction of highly effective ART in 1996, the incidence of dementia has fallen, but cases of mild NCI remain prevalent (Heaton et al., 2010).

During the last two decades, research has focused on estimating the prevalence of neuropsychological deficits and associated factors in HIV+ individuals, and interest in the effects of specific ART regimens on the CNS has increased. Magnetic resonance imaging (MRI), cerebrospinal fluid biomarkers, and neuropsychological assessments are used to explore differences between regimens.

The percentage of patients taking ART is high in developed countries such as Spain (Díaz et al., 2014). The study of well-treated patients was recently established as one of the main focuses of research on CNS involvement in HIV+ individuals (Spudich & Ances, 2015). Neuropsychological deficits seem to be less frequent in HIV+ patients receiving stable ART. To be off ART is a risk factor for earlier development of cognitive decline (Heaton et al., 2015). However, few studies with neuropsychological endpoints have been conducted in homogeneous cohorts of patients receiving effective treatment. For this reason, we performed comprehensive assessments including a wide range of neuropsychological measures in a selected sample of patients on ART.

Detailed analysis of neuropsychological functioning in aviremic patients can provide relevant information on the degree of disability expected among patients treated in our health system. Moreover, testing the impact of various ART regimens on brain and neuropsychological functioning could provide useful data on CNS safety and the risk of specific drugs.

The main objective of the present doctoral thesis was to expand current knowledge on neuropsychological functioning in HIV+ patients receiving effective ART in our health care context. In addition, we aimed to study the effect of protease inhibitor monotherapy on each of the neuropsychological measures applied.

The thesis is divided into five sections. In the first section (Introduction), we review general aspects of HIV infection and treatment, the more common types of neuropsychological dysfunction found in HIV+ patients, and the effect of ART on neurocognition. In the second section (Objective and hypothesis), we describe the specific objectives and premises that have led to our three empirical analyses. In the third section (Empirical analyses), we present the methods used in and the results obtained from each of the three analyses conducted. Briefly, Analysis 1 explored cognitive and motor abnormalities in the whole HIV+ sample, regardless of the type of ART. We also compared the neurocognitive and motor functioning of patients receiving protease inhibitors in monotherapy or as part of triple therapy, thus expanding knowledge on the neurological safety profile of protease inhibitor monotherapy. Analyses 2 and 3 explored for the first time the emotional processing based on the information contained in the face and voice of another person in a sample of long-term-treated HIV+ patients. The effect of protease inhibitor monotherapy on emotional processing was also tested. In the fourth section (Discussion), we debate the clinical relevance of our results in the context of care of HIV+ individuals, the neuropsychological processes and neural substrates potentially underlying our

outcomes, the limitations of our analyses, and future lines of research. Finally, in the fifth section (Conclusions), we present the conclusions obtained from our data.

1.2. Antecedentes y resumen: versión en Español

La infección por virus de inmunodeficiencia humana-tipo 1 (VIH) continúa teniendo alta prevalencia a nivel mundial y siendo un problema de salud pública (UNAIDS, 2013). Desde que Luc Montagnier aisló el virus en 1983 se han descrito casos de demencia asociada a la progresión de VIH en sistema nervioso central (SNC) (Navia et al., 1986). En los primeros años de epidemia de VIH, debido a la ausencia de tratamiento efectivo, el virus penetraba en SNC causando neuroinflamación y demencia. Desde que se introdujo la terapia antiretroviral (TAR) de alta eficacia en 1996, la incidencia de demencia asociada a VIH se ha reducido aunque los casos de deterioro cognitivo leves continúan siendo frecuentes (Heaton et al., 2010).

En las dos últimas décadas se ha tratado de estimar la prevalencia de déficits neuropsicológicos y los factores asociados con los déficits, en pacientes infectados por VIH. También ha crecido el interés por el estudio del efecto de regímenes de TAR específicos sobre el SNC. Para explorar las diferencias entre regímenes se utilizan resonancias magnéticas, biomarcadores de líquido cefalorraquídeo y evaluaciones neuropsicológicas.

En países occidentales como España, el porcentaje de pacientes que reciben TAR eficaz es alto (Díaz et al., 2014). Por ello, el estudio de pacientes que reciben TAR de manera estable se ha convertido en uno de los principales focos de investigación en el campo de neuro-VIH (Spudich & Ances, 2015). Los pacientes que reciben TAR eficaz parecen tener menores alteraciones neuropsicológicas que aquellos que no reciben TAR. De hecho, no recibir TAR es un factor de riesgo para el desarrollo de deterioro cognitivo (Heaton et al., 2015). Existen pocos estudios neuropsicológicos que se hayan llevado a cabo en pacientes en tratamiento eficaz. Por esa

razón, consideramos relevante realizar evaluaciones neuropsicológicas que incluyan un amplio número de medidas en muestras seleccionadas de pacientes en TAR eficaz. Un análisis exhaustivo del perfil neuropsicológico de los pacientes en TAR eficaz podría permitir obtener información sobre el tipo de discapacidad que pueden presentar muchos de los pacientes atendidos en nuestro sistema sanitario. Además, el estudio del impacto de diferentes regímenes antiretrovirales sobre el cerebro y las funciones neuropsicológicas en esta población puede aportar datos sobre su seguridad o riesgo.

Un objetivo general de la presente tesis doctoral fue ampliar la evidencia existente sobre el perfil de funcionamiento neuropsicológico de los pacientes infectados por VIH de que siguen TAR eficaz en nuestro entorno sanitario. También estudiamos el efecto de la monoterapia con inhibidores de la proteasa sobre las medidas neuropsicológicas aplicadas.

La presente tesis doctoral se compone de cinco secciones. En la primera sección (Introducción), revisamos los aspectos generales de la infección por VIH y sus tratamientos, los déficits neuropsicológicos más comunes en los pacientes infectados por virus y, los efectos del TAR en SNC. En la segunda sección (Objetivos e hipótesis), describimos los objetivos específicos y las premisas de las que parten nuestros análisis empíricos. En la tercera sección (Análisis empíricos), presentamos los métodos y resultados de cada uno de los análisis realizados. Resumiendo, en el Análisis 1 exploramos el perfil de alteraciones cognitivas y motoras en la muestra completa de pacientes, con independencia del tipo de TAR recibido. Además comparamos el funcionamiento cognitivo y motor de los pacientes dependiendo del tipo de TAR recibido -inhibidores de la proteasa en monoterapia o en terapia triple-, con el objetivo de generar nuevos datos sobre la seguridad del uso de inhibidores de la proteasa en monoterapia en SNC. En los Análisis 2 y 3 estudiamos, por primera vez en una muestra de pacientes que habían recibido TAR eficaz durante un largo periodo de tiempo, el procesamiento emocional realizado sobre la información

contenida en las caras y voces de otras personas. También analizamos el efecto de la monoterapia con inhibidores de proteasa sobre los procesos estudiados. En la cuarta sección (Discusión) reflexionamos sobre la relevancia clínica de nuestros resultados en el contexto clínico de la infección por VIH, los procesos neuropsicológicos y las bases neuronales que podían estar relacionados con nuestros resultados, las limitaciones de nuestros análisis y futuras líneas de investigación. Finalmente, en la sección quinta (Conclusiones) resumimos las conclusiones obtenidas desde nuestros datos.

2. INTRODUCTION

2.1. Human Immunodeficiency Virus in the body

HIV is a retrovirus that infects human cells expressing CCR5 and CXCR4 receptors, primarily CD4+ T lymphocytes (CD4), resulting in progressive impairment of the immune response (The Centers for Disease Control, 1993). Once virions enter the lymphocyte, the virus replicates using the cellular mechanisms of CD4 cells, which eventually die (Moss & Bacchetti, 1989). As the CD4 count decreases with viral replication, the risk of life-threatening conditions (severe immunodeficiency, serious opportunistic infections, and cancer) increases (The Centers for Disease Control, 1993). While healthy adults have between 500 and 1,500 CD4 cells per cubic millimeter of blood, HIV+ people may have much lower counts. A higher risk of disease progression has been also associated with the patient's CD4 nadir, ie, the lowest CD4 count recorded (Miller et al., 1999).

Different stages of progression may be observed during the course of the infection. The acute stage begins within 2-4 weeks after infection, with symptoms similar to those of influenza. The clinical latency or asymptomatic stage usually follows the acute stage and is characterized by good immune function and absence of symptoms. Long-term treatment also enables the patient to remain in the asymptomatic stage. Finally, the most advanced stage of infection is the acquired immunodeficiency syndrome (AIDS) (Trilla, Mensa, & Gatell, 1988). The revised definition of the Centers for Disease Control and Prevention considers AIDS to be a CD4+ nadir < 200 cells/mm^3 or clinical conditions associated with immunosuppression, such as opportunistic infections and malignancies (The Centers for Disease Control, 1993). In the absence of treatment, most patients (80-90%) are diagnosed with AIDS within 7-12 years of infection

(Mandell, 2009) while a smaller number progress faster (5%) (Mellors et al., 1997) or slower (4-7%) (Poropatich & Sullivan, 2011).

Pharmacological treatments prevent progression of HIV and death. Treatment controls viral replication and restores the immune function damaged by the virus. The drugs used during the first decade of the HIV epidemic were unable to control viral replication, and millions of people died. Since 1996 the implementation of highly effective ART dramatically reduced the morbidity and mortality associated with HIV infection (Powderly, 2002). Effective ART regimens prevent viral replication in peripheral fluids. HIV RNA (ribonucleic acid) copies are not detectable in blood samples (ie, levels below 50 copies/milliliter), and patients are considered aviremic or virologically suppressed. Despite its efficacy, ART does not eradicate the virus, because it is unable to act in resting cells ("HIV reservoirs"). Therefore, if patients stop taking ART, viral replication and destruction of the immune system start afresh. Treatment should be taken daily, and good adherence is basic for control of viremia. Loss of adherence leads to reactivation of viral replication.

The antiretroviral armamentarium comprises more than 30 drugs that are available for prescription in combination depending on the clinical characteristics of the individual patient. While the need to start ART based on the CD4 count has been a matter of debate in recent years, the latest findings from the START clinical trial have led experts in developed countries to modify their recommendations. From now on, all patients should be treated regardless of their CD4 count (INSIGHT START Study Group et al., 2015). In addition to controlling disease progression, effective ART prevents new infections, because it stops transmission of HIV to others (Hull & Montaner, 2013).

Access to ART is not free in all countries. Only 8 million of the 34 million HIV+ people worldwide have access to treatment. In Spain, there are between 140,000 and 170,000 HIV+ people

(prevalence of 0.3%) (UNAIDS, 2013). Access to ART is free for all patients through the public health system. Here, about 74% of people diagnosed with HIV infection follow proper medical monitoring, and roughly 94% are receiving ART. An estimated 93% of treated patients in Spain achieve viral control (Díaz et al., 2014).

The virus is transmitted when a vehicle such as blood, semen, vaginal fluid, or breast milk from an HIV+ person comes into contact with the mucous membranes or damaged tissues of a non-HIV+ person. The most common modes of transmission are unprotected sex, shared needles, and mother-to-child transmission during pregnancy or birth. The prevalence of a specific mode of transmission differs depending on demographic factors. In Spain, the most predominant mode of transmission in new infections is anal intercourse between men who have sex with men (42.5%) followed by unprotected heterosexual intercourse (34.5%). However, parenteral transmission is still the most prevalent mode in Spain (30-50%) owing to the epidemic of parenteral drug use in the 1980s and 1990s (Díez et al., 2012). Because most people who were infected by HIV through parenteral drug use are also co-infected with hepatitis C virus (HCV), Spain has one of the highest frequencies of HIV/HCV co-infection (between 60,000 and 80,000 people) (González-García et al., 2005).

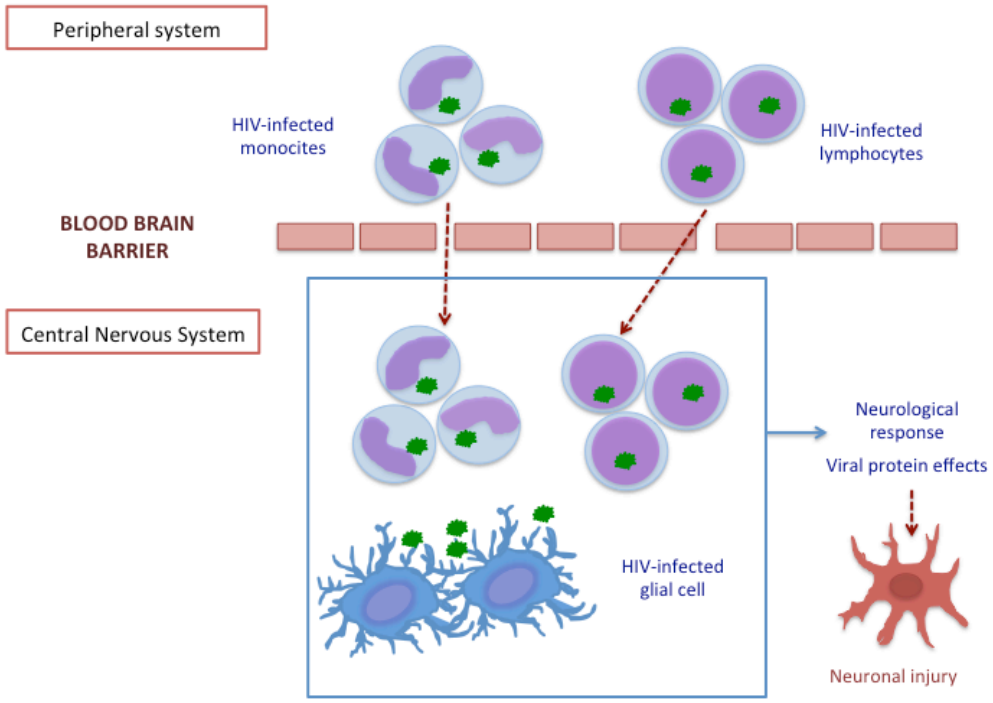
Therefore, since the clinical conditions of HIV+ patients differ considerably, interventions should be managed on an individual basis. In addition, research is affected by the heterogeneous nature of clinical findings, with the result that most studies are based on clinically similar patients. Selection is essential if we are to draw robust conclusions about the role of HIV infection and treatment on the different body systems.

2.2. Human immunodeficiency virus and neurocognition

HIV can infect the CNS and impair its function. The virus is thought to cross the blood-brain barrier through infected monocytes and lymphocytes (Valcour, Sithinamsuwan, Letendre, & Ances, 2010). HIV enters glial cells and produces inflammation of the CNS (Vance, Fazeli, Grant, Slater, & Raper, 2013). In addition, glial cell death leads to secretion of inflammatory molecules such as cytokines, which promote oxidative stress (Fields, 2009). Although HIV does not kill neurons directly, molecules resulting from the neurological immune response and the effects of viral proteins may injure them (Valcour et al., 2010). After crossing the blood-brain barrier, HIV virions travel through cerebrospinal fluid and accumulate in the ventricles. From there, HIV primarily affects the integrity of the subcortical and fronto-striatal systems (Paul et al., 2007; Pfefferbaum et al., 2009). However, reductions in other cortical areas have been observed (Thompson et al., 2005). Brain dysfunction caused by HIV triggers neuropsychological deficits consistent with relatively selective impairment in fronto-striatal circuits (Heaton et al., 1995; White, Heaton, & Monsch, 1995). Figure 1 summarizes the potential process of HIV damage on the brain.

Penetration of the CNS by HIV produces progressive brain encephalitis. In the absence of ART, the disease progresses, leading to severe dementia and death (Levy, Bredesen, & Rosenblum, 1985). Before the advent of ART, the prevalence of dementia associated with HIV encephalitis was approximately 16% (McArthur et al., 1993). However, effective ART controls replication of HIV in the brain and reverses dementia associated with HIV-induced encephalitis. At present, the frequency of severe dementia has fallen to below 2% (Heaton et al., 2010). Furthermore, effective ART stops HIV trafficking towards the CNS and increases the CD4 count. Thus, effective regimens reduce the risk of independent HIV replication in the brain parenchyma (“HIV compartmentalization”) (Ellis, Gamst, Capparelli, Spector, & Hsia, 2000).

Figure 1. Potential mechanisms of CNS infection by HIV: schematic overview (modified from Valcour et al. 2010)



Although the number of cases of dementia has fallen considerably, significant frequencies of less severe neuropsychological disorders persist among HIV+ people (Cysique, Maruff, & Brew, 2004a; Heaton et al., 2010; 2011; Sacktor et al., 2002). Deficits persist even in aviremic patients receiving effective ART (Cysique & Brew, 2011a; Garvey, Surendrakumar, & Winston, 2011). Persistent deficits have been associated with irreversible brain damage produced during previous episodes of advanced immunosuppression (Heaton et al., 2010). It also seems plausible that the chronic cellular activation occurring after the virus has penetrated the CNS causes nerve cell dysfunction, regardless of subsequent effective ART (Clifford, 2008). Moreover, cases of HIV compartmentalization in the CNS that prevent viral suppression in the brain have been described in a small proportion of patients despite the peripheral effectiveness of ART (Ferretti, Gisslén, Cinque, & Price, 2015).

The neuropsychological impairment caused by HIV has been termed HIV-associated neurocognitive disorders (HAND). In 2007, the American Association of Neurology (ANN) defined the diagnostic criteria for HAND based on an expert panel consensus (Antinori et al., 2007). To detect and classify different types of HAND, authors recommend using comprehensive neuropsychological test batteries covering at least five of the following commonly impaired domains: verbal/language; attention/working memory; abstraction/executive functions; memory (learning and recall); speed of information processing; sensory-perceptual skills; and motor skills. In addition, at least two test measures per domain are recommended. Different categories are established depending on the severity of alterations and degree of limitations to daily functioning. The categories proposed in ascending order of severity are HIV-associated asymptomatic neurocognitive impairment (ANI), HIV-associated mild neurocognitive disorder (MND), and HIV-associated dementia (HAD). Specific criteria for each category are summarized in Table 1. Cases of delirium or impairment fully explained by other causes ("confounding conditions") are excluded. Evidence that HIV infection caused the deficits or an exclusion diagnosis is necessary to confirm the presence of HAND. If medical information is not available, the diagnosis is not HAND but NCI. Because not all studies conduct the necessary medical assessment for the diagnosis of HAND, all further references in the text are to NCI.

The different frequencies of NCI found in heterogeneous cohorts of HIV+ people could be due to the impact of several clinical features. Lower frequencies of NCI (18-21%) in patients with few comorbidities (Cysique & Brew, 2011a; Garvey et al., 2011) contrast with frequencies of up to 83% in those with other confounding conditions (Heaton et al., 2010). Moreover, patients with confounding conditions had a higher risk of cognitive decline over time (Heaton et al., 2015). The confounding conditions in the HIV+ population include traumatic brain injury with functional decline, mental disability, ongoing substance use disorders, current opportunistic CNS infections

(eg, meningitis, brain tumours, epilepsy), and decompensated liver disease due to HCV (Antinori et al., 2007).

Markers of disease progression have been also associated with NCI. While 55% of HIV+ people with advanced disease have NCI, the frequency could decrease to 30% in those at earlier stages of the infection (Heaton et al., 1995). Similarly, higher frequencies of NCI are detected in patients with a lower CD4 nadir (Ellis et al., 2011; Heaton et al., 2010; Muñoz-Moreno et al., 2008). Moreover, data suggest that ART can protect against HIV-induced brain damage. Aviremic HIV+ patients receiving effective ART have the lowest frequencies of NCI (18-35%) (Cysique & Brew, 2011a; Cysique, Maruff, & Brew, 2004b; Garvey et al., 2011). Conversely, patients who are off ART experience earlier cognitive decline over time (Heaton et al., 2015).

Other, non-clinical factors have been associated with NCI among HIV+ individuals. Older patients seem to have higher frequencies of NCI (Cherner et al., 2004; Sacktor et al., 2007; Valcour et al., 2004). Moreover, HIV+ patients with lower premorbid global functioning estimated by intelligence quotient (IQ) had higher frequencies of persistent neurocognitive deficits (Cysique & Brew, 2011a). Those with a better premorbid estimated IQ experienced greater cognitive improvement over time (Heaton et al., 2015). Data for HIV+ people are compatible with the cognitive reserve hypothesis, which postulates that intellectual capacity can compensate brain damage. Intellectual stimulation such as development of literacy skills might change the organization of the brain, thus increasing protection against cognitive decline (Manly, Touradji, Tang, & Stern, 2003).

In summary, several factors affect the probability of experiencing NCI. Older patients, patients who are off ART, patients with neurological comorbidities, patients with markers of HIV disease progression, and those with low cognitive reserve might have the highest frequencies of impairment.

Table 1. Outline of criteria for classifying HIV-associated neurocognitive disorders (HAND) (adapted from Antinori et al. 2007)

HAND CATEGORY	NEUROPSYCHOLOGICAL EVALUATION	DAILY FUNTIONAL IMPAIRMENT
ANI	Neurocognitive/motor impairment in ≥ 2 domains (>1 SD below a demographically appropriate normative mean), that cannot be explained by comorbid condition*	Not reported or demonstrated
MND	Neurocognitive/motor impairment in ≥ 2 domains (>1 SD below a demographically appropriate normative mean), that cannot be explained by comorbid condition*	Mild functional decline reported or demonstrated
HAD	Neurocognitive/motor impairment in ≥ 2 domains (>2SD below a demographically appropriate normative mean), that cannot be explained by comorbid condition*	Major functional decline reported or demonstrated

ANI: Asymptomatic neurocognitive impairment. MND= Mild neurocognitive disorder. HAD= HIV- associated dementia. SD= Standard deviation *Comorbid condition includes: opportunistic central nervous system disease, systemic illness, psychiatric illness, substance use disorders, or medications with central nervous system effects.

2.3. Antirretroviral therapy, monotherapy and central nervous system effects

Despite the decreased frequency of NCI observed in HIV+ people receiving effective ART, neuropsychological disorders have not completely disappeared. The reasons for persistent deficits are unknown. In addition to the explanations described above (previous irreversible brain dysfunctions, chronic cellular activation, or the compartmentalization of the virus in the brain) (Clifford, 2008; Ferretti et al., 2015; Heaton et al., 2010), recent hypotheses point to the effects of specific antiretroviral drugs on brain functioning.

Lentendre (2011) proposed a conceptual therapeutic window for the effectiveness of ART in the CNS. The reduced ability to penetrate the CNS could lead to brain damage associated with HIV viral replication and immune activation, whereas excessive CNS drug concentrations might cause

drug-related neurotoxic effects. During the last decade, several studies have tried to detect the effects of ART regimens with suboptimal CNS penetration or excessive neurotoxicity.

Some antiretroviral drugs are considered neuroactive whereas others are not (Table 2). The neuroactive drugs are able to penetrate the blood-brain barrier and inhibit viral replication in CNS. In order to classify antiretroviral drugs according to the ability to act in the CNS, a CNS penetration effectiveness (CPE) score was assigned to each one of the antiretroviral drugs studied. A ranking was proposed in 2008 (Letendre, 2008) and reviewed in 2010 (Letendre et al., 2010). In the absence of direct measures, estimates of the ability of specific antiretroviral drugs to penetrate the CNS were based on molecular features such as size or fat solubility. In addition, pharmacokinetic and pharmacodynamic properties such as the drug's ability to inhibit viral replication in cerebrospinal fluid were used as estimators (Letendre, 2011). In ART regimens comprising several antiretroviral drugs, the CPE scores for each drug were totalled. A higher CPE score corresponded to a higher theoretical ability to penetrate the CNS (Table 2).

The clinical relevance of the CPE score is open to debate. Some studies have demonstrated an association between higher CPE scores and better CNS markers of functioning, whereas others have not (for a review see Cysique, Waters, & Brew, 2011b). In vitro experiments have shown that some ART regimens produce neural damage at the concentrations reached in the CNS (Robertson, Liner, & Meeker, 2012). Neurotoxic effects due to increased CNS penetration by ART were suggested in the ACTG 736 clinical trial, which found poorer performance to be associated with ART regimens with higher CPE scores in specific neurocognitive measures (Marra et al., 2009). A recent analysis found that the risk of HIV dementia was higher in naïve patients who initiated ART regimens with high CPE scores (Caniglia et al., 2014). Moreover, only one antiretroviral drug—efavirenz—has shown clinically relevant neurotoxic symptoms. Patients receiving regimens containing efavirenz had higher frequencies of neurocognitive dysfunction in

a small cross-sectional study (Ciccarelli et al., 2011). No other antiretroviral drug has yet led to clinical symptoms suggestive of scarce or excessive penetration of the CNS. Table 2 describes the most common drug combinations and summarizes the CPE classification system.

Table 2. Overview of antiretroviral drug and central nervous system penetration (adapted from Lettendre 2010)

TYPE OF ANTIRRETROVIRAL DRUG		CPE SCORE
Nucleoside analogue reverse transcriptase inhibitors (NARTIs)	Abacavir	3
	Emtricitabine	3
	Lamivudine	2
	Tenofovir	1
Non nucleoside analogue reverse transcriptase inhibitors (NNRTIs)	Nevirapine	4
	Efavirenz	3
	Etravirine	2
Protease inhibitors (PI)	Darunavir/ritonavir (DAR/r)	3
	Lopinavir/ritonavir (LOP/r)	3
	Atazanavir/ritonavir	2
	Atazanavir	2
Entry/fusion inhibitors (FI)	Maraviroc	3
	Enfuvirtide	1
Integrase inhibitors (II)	Raltegravir	3

Grey shading represents drugs that have demonstrated effective peripheral viral suppression in monotherapy. Standard triple therapy regimens include several drug combinations, the most common: 2 NARTIs+1 NNRTIs or PI or II. The Picasso Study included: DAR/r or LOP/r in monotherapy or 2NARTIs+ 1PI (DAR/r or LOP/r) in triple therapy. Central nervous system penetration effectiveness (CPE) score estimates the ability to penetrate the CNS: 1=below average; 2=average; 3= above average; 4= much above average. Drugs with CPE$\geq$ 3 are considered neuroactive drugs.

Since the introduction of ART, the standard regimens combine at least three antiretroviral drugs. Also protease inhibitor monotherapy has been prescribed in simplification strategies. However, this approach is only indicated in patients who have previously achieved suppression of plasma viral load with a triple therapy regimen. Switching treatment aims to reduce drug toxicity and costs. Monotherapy enables most patients to maintain peripheral viral suppression

(Oddershede, Walker, Paton, & Stöhr, 2014; Arribas et al., 2010; Katlama et al., 2010; Paton et al., 2015; Pulido et al., 2008). However, there is no consensus on the safety of simplifying to monotherapy. European and Spanish guidelines allow simplification from triple therapy to monotherapy, whereas American guidelines do not (Expert Panel of European AIDS Clinical Society, 2014; Expert Panel of GESIDA and the National AIDS Plan, 2015; Panel on Antiretroviral Guidelines for Adults and Adolescents, 2009). Poor CNS penetration of protease inhibitors is one of the reasons proposed by the International Antiviral Society–USA panel for not recommending monotherapy (Thompson et al., 2012).

As shown in Table 2, both boosted lopinavir and darunavir monotherapy regimens have a CPE score of 3, including only one neuroactive drug. Conversely, triple therapy regimens have CPE scores of between 6 and 9 and usually include one or two neuroactive drugs. In contrast with the theoretically poor CNS penetration proposed, studies with neurocognitive endpoints in aviremic HIV+ patients did not show worse outcomes in patients receiving monotherapy than in those receiving standard triple therapy (Santos et al., 2013; Winston, Arenas-Pinto, Stöhr, & Fisher, 2013; Bunupuradah et al., 2012; Clarke et al., 2014).

2.4. Emotional impairment related to brain dysfunctions in HIV+ individuals

Specific variables appear to be involved in the etiology of affective symptoms in HIV+ individuals compared with the general population. Problems in coping with the disease, loss of social support, stigmatization, and discrimination have all been associated with affective symptoms (White et al., 2012). In addition, disruption of the prefrontal cortex and paralimbic brain structures by HIV infection are thought to produce affective symptoms (McIntosh, Rosselli, Uddin, & Antoni, 2015). High frequencies of affective symptoms have also been described in

conditions that affect emotion-related brain systems such as Parkinson disease and Huntington disease (Péron, Dondaine, Le Jeune, Grandjean, & Vérin, 2012; Van Duijn et al., 2014). Frequent depressive disorder, apathy, alexithymia, and impaired recognition of facial emotions have been described in HIV+ individuals.

Depressive symptoms assessed using self-administered questionnaires appear systematically among HIV+ people from different countries (15.7%) (Robertson, Bayon, Molina, & McNamara, 2014). In addition, interview studies based on the criteria of the Diagnostic and Statistical Manual of Mental Health Disorders (DSM-IV) reported that mood disorders (Pence, Miller, Whetten, Eron, & Gaynes, 2006), particularly depression (Bayon et al., 2014) were common in HIV+ individuals (32% and 21% respectively).

Consistent with the overall protective role of treatment in neurocognition, a study of a large Spanish cohort revealed that patients on ART developed fewer episodes of significant depression (Gutiérrez et al., 2014). According to the neurotoxic effects of efavirenz on cognition, regimens containing this drug have been associated with depressive symptoms, including suicidality (Mollan et al., 2014) and sleep disturbances (Allavena et al., 2014).

Apathy is an affective symptom that could be associated with specific neurological dysfunctions in HIV+ patients. It is defined as a quantitative reduction in self-generated purposeful behaviours or lack of motivation (Levy, 2012). Apathy has been commonly observed in patients with circuit dysfunction in the frontal lobe, basal ganglia, or frontal-basal ganglia (Levy & Dubois, 2006). The propensity of HIV to replicate in frontal-striatal circuits (McIntosh et al., 2015) might account for the high frequencies of self-referred apathy found in HIV+ individuals (30-50%) (Castellon, Hinkin, & Myers, 2000; Castellon, Hinkin, Wood, & Yarema, 1998; Kamat et al., 2015; Shapiro, Mahoney, Zingman, Pogge, & Verghese, 2013). Several studies have also found diminished performance in HIV+ individuals with high degrees of apathy in cognitive tasks that are sensitive

to fronto-subcortical dysfunctions (Castellon et al., 1998; 2000; Cole et al., 2007; Paul, Flanigan, & Tashima, 2005b; Shapiro et al., 2013). It is believed that disruption of frontal-subcortical circuits might be a common cause of apathy and of dysfunction in executive functions and processing speed (Castellon et al., 2000; Shapiro et al., 2013). Frontal white matter abnormalities (Hoare et al., 2010; Kamat et al., 2014) and reduced volume of basal ganglia (Paul et al., 2005a) have been found in HIV+ patients with high apathy levels, thus supporting the brain damage hypothesis in affective impairment.

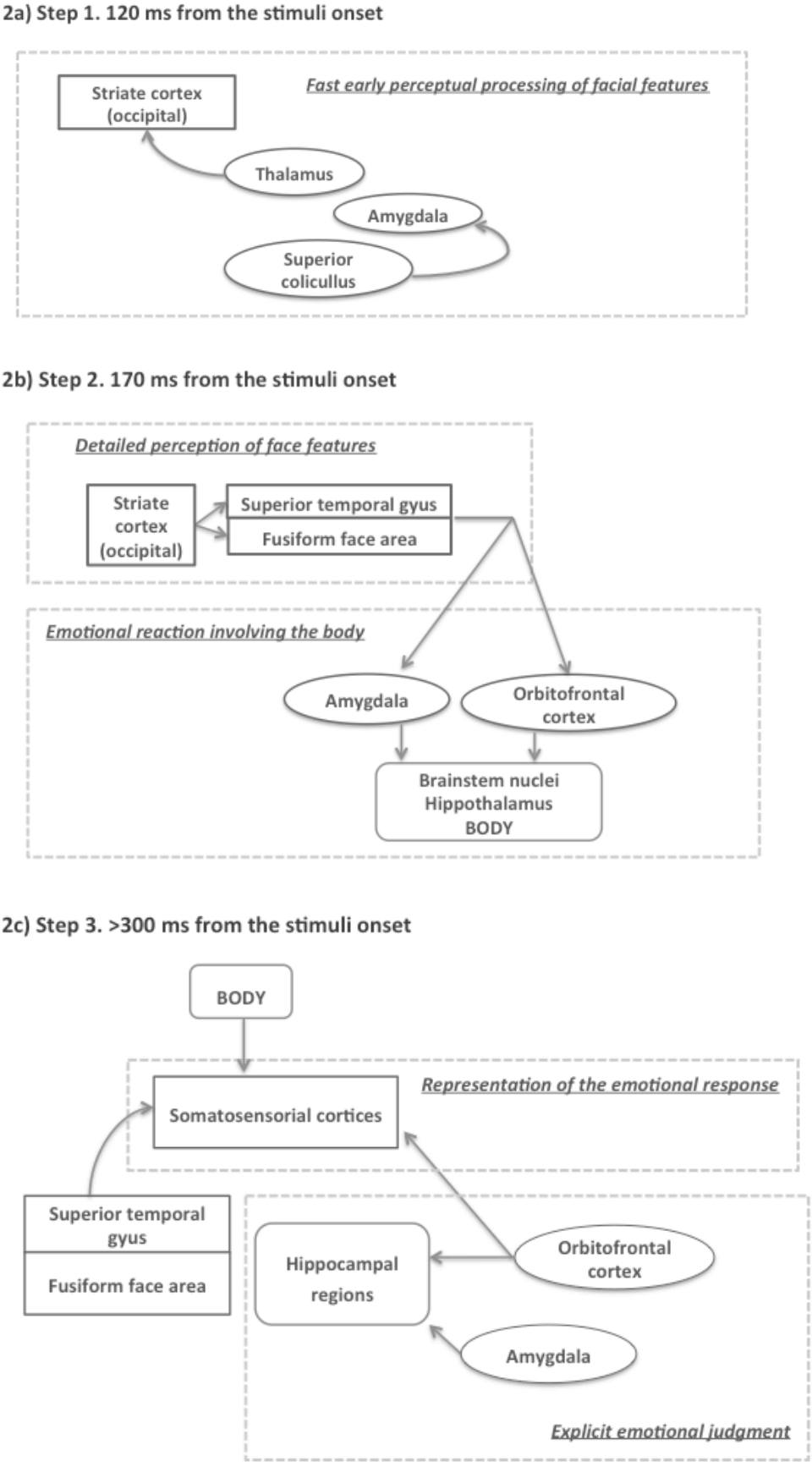
Alexithymia, in which patients experience difficulty identifying and describing feelings, is an emotional syndrome that has become increasingly frequent among HIV+ individuals (Bagby & Taylor, 1997). One small study found higher frequencies of self-referred alexithymia in a group of HIV+ patients than in seronegative individuals (20% vs. 0%) (Bogdanova, Díaz-Santos, & Cronin-Golomb, 2010). Patients with alexithymia also had diminished performance in cognitive tasks that are sensitive to fronto-subcortical dysfunctions. The authors suggest that alexithymia deficits may be a consequence of the effect of HIV infection on fronto-striatal systems (Bogdanova et al., 2010) (McIntosh et al., 2014).

The ability to recognize emotions from the information expressed on the faces of other people has been found to be impaired in several cohorts of HIV+ patients (Baldonero et al., 2013; Clark, Cohen, Westbrook, Devlin, & Tashima, 2010; Lane, Moore, Batchelor, Brew, & Cysique, 2012). Fear recognition deficits were associated with reduced anterior cingulate cortex volumes in one of these samples (Clark et al., 2015). Patients with specific emotional processing deficits had also diminished performance in tests that are sensitive to fronto-subcortical dysfunctions (working memory, speed of information processing, and fine motor skills) (Lane et al., 2012).

Perception and recognition of facial emotion depend on neural systems composed of cortical and subcortical structures (Haxby, Hoffman, & Gobbini, 2000). Adolphs (2002) proposed a multi-

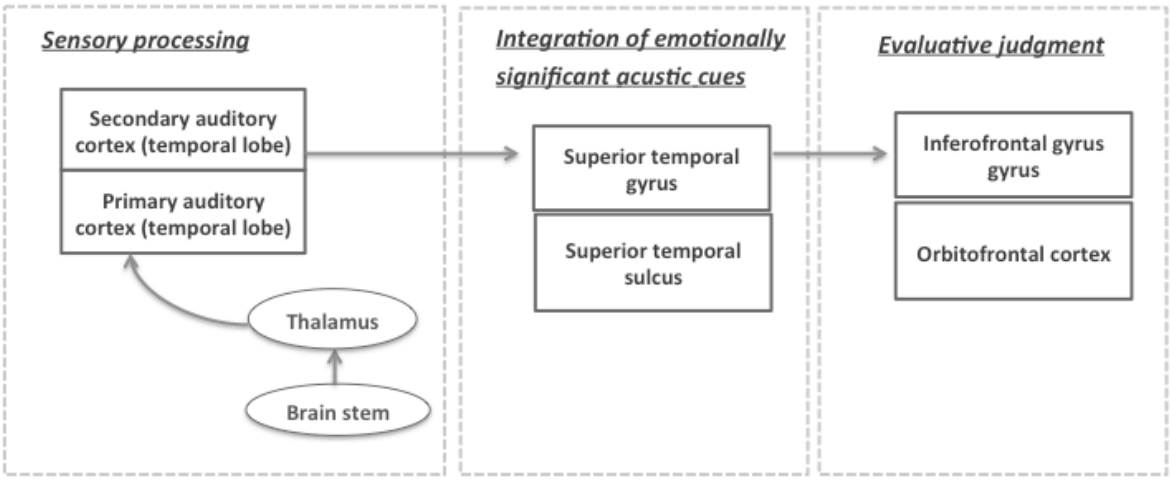
step model to explain the neural substrate of facial emotion processing. First, visual information travels from the superior colliculus and the pulvinar thalamus through the amygdala and the occipital striate cortex to enable early perceptual processing (Figure 2a). Information then travels up to other occipito-temporal visual cortices, generating detailed perceptions that make it possible to distinguish emotional expressions from facial features. Simultaneously, emotional reactions in the body of the observer result from the connection of the amygdala and orbito-frontal cortex with motor structures, the hypothalamus, and brainstem nuclei (Figure 2b). The somatosensory cortices subsequently build a representation of this emotional response. Finally, the amygdala and orbito-frontal cortex retrieve associated knowledge about emotion through connections with other cortical and hippocampal regions, thus enabling explicit recognition of expression of facial emotion (Figure 2c).

Figure 2. Neural systems for recognizing facial emotions (modified from Adolphs 2002).



In addition, emotions in others may be recognized from their voice prosody. Vocal emotion processing also requires proper functioning of various brain systems. Schirmer and Kotz (2006) proposed a model to explain the successive steps required for vocal emotion recognition. First, the acoustic information runs from the ear to the brain stem up to the thalamus. This information then reaches the primary and secondary auditory cortex (temporal area), where acoustic information is analysed and integrated in complex tones. After that, the superior temporal gyrus and superior temporal sulcus synthesize emotionally salient information travelling towards prefrontal areas to enable explicit evaluative judgment. Other subcortical structures, such as the amygdala or basal ganglia, are involved in facilitating subprocesses associated with context relevance (Figure 3).

Figure 3. Neural systems of voice emotion processing (modified from Schirmer& Kotz 2006).



Objective neuropsychological tests have been used in clinically heterogeneous HIV+ cohorts to explore perception and recognition of facial emotion (Baldonero et al., 2013; Clark et al., 2010; Lane et al., 2012). To our knowledge, none were conducted in cohorts of long-term virologically suppressed HIV+ patients. Moreover, recognition of emotional tone of voice (emotional prosody) in other people has not been previously explored in HIV+ patients.

3. OBJECTIVES AND HYPOTHESIS

The present doctoral thesis aims to expand current knowledge on the neuropsychological patterns experienced by long-term treated aviremic HIV+ patients. In particular, we aim to determine the frequency of cognitive, motor, and emotional processing dysfunction in these patients. We also wanted to test the effect of using protease inhibitors in monotherapy or as part of triple therapy on the neuropsychological outcomes found.

Specific objectives and hypotheses are described separately for each empirical analysis:

Analysis 1: Pattern of cognitive and motor functioning in aviremic HIV+ patients and effect of receiving boosted protease inhibitor monotherapy

Main objectives and hypothesis: to compare two groups of HIV+ patients receiving different types of ART regimens by assessing a series of cognitive and motor neuropsychological measures. The regimens used were protease inhibitors in monotherapy or administered as triple therapy with two additional nucleoside reverse transcriptase inhibitors. To explore the effect of monotherapy and triple therapy on patients with NCI. Although the frequency of NCI did not differ by treatment group in our sample in a previous analysis (Pérez-Valero et al., 2013), differences in specific cognitive and motor processes cannot be ruled out. We did not formulate an a priori hypothesis owing to the lack of detailed neuropsychological analyses in patients receiving protease inhibitor monotherapy.

Secondary objective and hypothesis: to explore the pattern of cognitive and motor functioning in our whole sample of aviremic HIV+ patients, regardless of the type of ART. To our knowledge, no studies have previously described the profile of neuropsychological deficits in an HIV+ sample on effective long-term ART. Based on the only study that included exclusively aviremic patients, we expected deficits to be most frequent in verbal memory and attention/working memory tasks

(Ciccarelli et al., 2013). Frequent deficits in other tasks cannot be ruled out based on differences between test batteries and samples.

Analysis 2: Facial emotion processing deficits among long-term aviremic HIV+ patients and effect of receiving boosted protease inhibitor monotherapy

Main objective and hypothesis: to determine whether our sample of long-term virologically suppressed HIV+ patients had facial emotion recognition deficits similar to those reported in cohorts of clinically different HIV+ patients (Baldonero et al., 2013; Clark et al., 2010; Lane et al., 2012). Since all patients were receiving ART in the study by Lane et al (2012), our hypothesis was formulated based on their results. We expected preservation of facial recognition processing but deficits in recognition for particular basic emotions.

Secondary objectives: to explore deficits in discrimination and memorization of facial expressions, as well as the effect of medical variables or global neurocognitive status on facial emotion processing. We postulated different hypotheses for each objective. Based on the results of all the studies published in the field, we expected no facial discrimination deficits. We did not postulate an a priori hypothesis about memory tasks performance, because prior studies did not use facial memory tasks.

Our hypothesis regarding the impact of NCI status is also based on prior outcomes of patients on ART (Lane et al., 2012). We expected HIV+ patients with NCI to have recognition deficits, in contrast with patients who did not have NCI.

Based on the results of Lane et al. (2012), we postulated a hypothesis on the effect of medical variables on facial emotion processing. We did not expect any association between HIV markers of progression such as CD4+ nadir or AIDS status and facial emotion processing deficits. A

hypothesis on the impact of HCV co-infection or the use of protease inhibitors in monotherapy was not established owing to the lack of previous results.

Analysis 3: Recognition of emotional tone of voice (emotional prosody) in long-term aviremic HIV+ patients and effect of receiving boosted protease inhibitor monotherapy

Main objective: to explore the brain imaging correlates (assessed using 3-T MRI and spectroscopy [MRS]) of a newly designed vocal emotion processing test in aviremic HIV+ individuals receiving ART. We were also interested in determining whether correlations persisted, regardless of NCI.

Secondary objective: we aimed to explore whether our selected sample of HIV+ patients had worse performance in the vocal emotion processing test than a group of healthy adults.

We hypothesized that poorer levels of cerebral markers might be associated with poorer prosody test performance. HIV infection can affect subcortical and fronto-striatal structures (Paul et al., 2007; Pfefferbaum et al., 2009), and several subcortical and fronto-temporal structures support emotional prosody processing (Schirmer & Kotz, 2006). Moreover, deficits in emotional prosody processing are plausible because they have also been described in conditions such as Parkinson disease, in which subcortical and fronto-striatal systems can be altered (Buxton, MacDonald, & Tippett, 2013). We did not formulate more specific hypotheses owing to the exploratory and novel character of the study. To our knowledge, this is the first study of emotional prosody processing in a sample of HIV+ individuals.

4. EMPIRICAL ANALYSES

4.1. Analysis 1. Pattern of cognitive and motor deficits in aviremic HIV+ patients and effect of receiving boosted protease inhibitor monotherapy

4.1.1.Methods

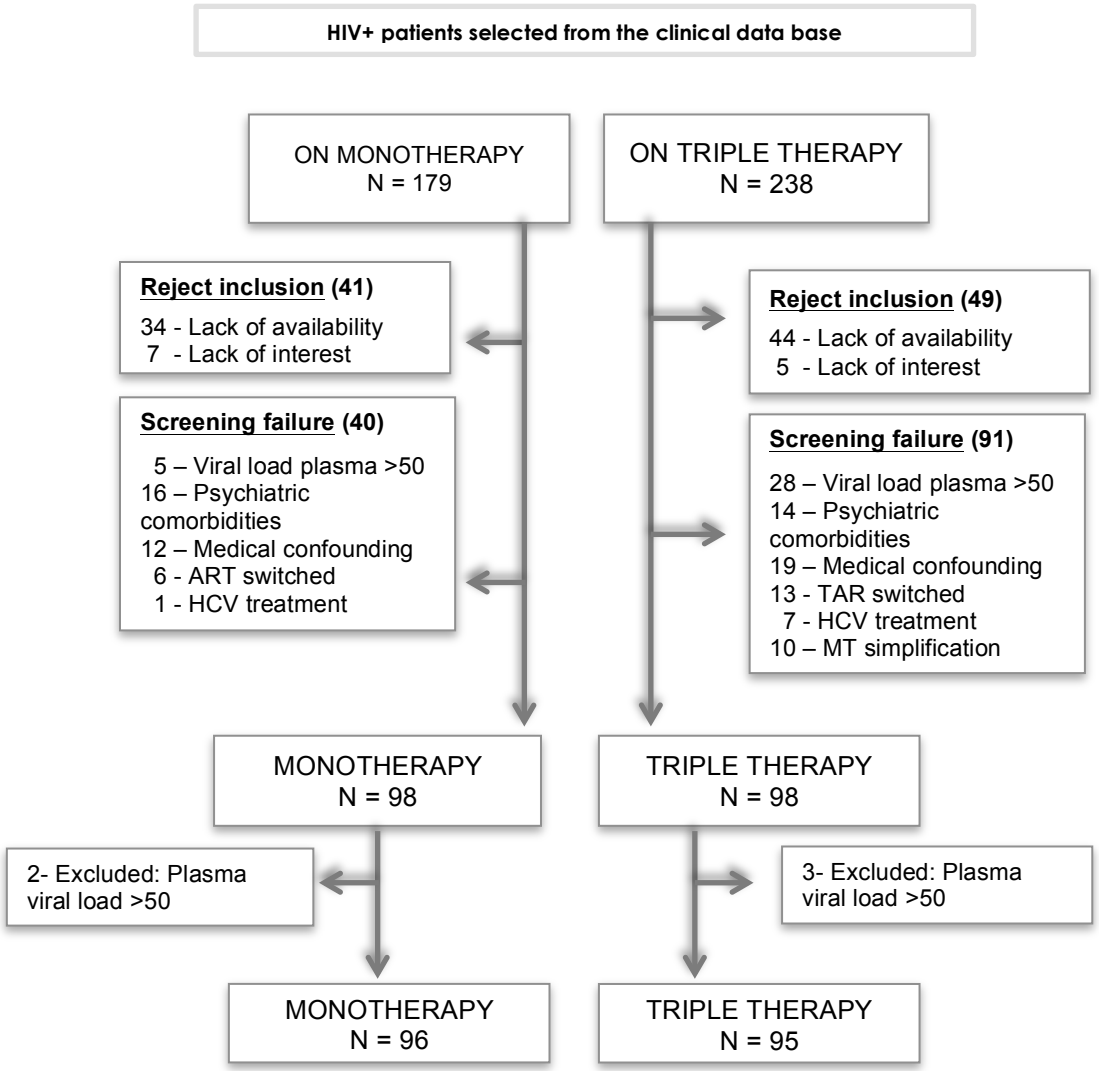
The present analysis is a sub-analysis of the Picasso Study, a parent study that compared rates of NCI in patients treated with protease inhibitors in monotherapy and as part of triple therapy regimens. In this section we described methods previously reported in Perez-Valero et al. (2013) and González- Baeza et al. (2014). Also, the results have been previously published in González-Baeza et al. (2014).

Participants

We selected patients receiving lopinavir/ritonavir or darunavir/ritonavir in monotherapy (monotherapy group) or as part of triple therapy with two nucleoside/nucleotide reverse transcriptase inhibitors (triple therapy group). At inclusion all had at least one year of virological suppression. Virological suppression was defined as two measurements of plasma HIV RNA below 50 copies/millilitre. A single virologic rebound of 50–500 HIV RNA copies/millilitre ("single blip") was allowed in the year prior to the inclusion. Patients were excluded if they had active CNS opportunistic disease, global intellectual disabilities, substance abuse during the previous three months, alcohol abuse during the six previous months, diagnosis of psychotic disorders according to the DSM-IV, neuromuscular disorders, visual dysfunctions or use of psychiatric medications which could interfere with results of the neurocognitive evaluation. Patients who were receiving triple therapy at inclusion, but had previously received protease inhibitor monotherapy for at least one year were excluded. Also we excluded patients on active

treatment of HCV and those who had finished the treatment during the previous six months. The study was systematically offered during the recruitment period (April 2011 to June 2012) to all patients who fulfilled all the inclusion and none of the exclusion criteria. Participants were recruited from the HIV Unit of the Hospital Universitario La Paz and Hospital Universitario Doce de Octubre (Madrid, Spain). Rates of participants included and reasons for exclusion are summarized in the Figure 4.

Figure 4. Picasso Study process flow chart (modiffied from Pérez-Valero et al. 2013)



We identified 417 potential study candidates. Finally we included 191 patients who completed the basal visit. A neuropsychologist blinded to the treatment group conducted a comprehensive neuropsychological battery. In addition, all participants completed medical and laboratory assessments. A medical doctor captured medical and laboratory measures from the patients' records.

Ethics Statement

This study and its procedures were conduced according with the principles expressed in the Declaration of Helsinki. The local Ethics Committees for Clinical Research of each participant hospital - Comité Ético de Investigación Clínica del Hospital Universitario La Paz de Madrid and Comité Ético de Investigación Clínica del Hospital Universitario Doce de Octubre de Madrid. Also the Institutional review boards of both hospitals - Comisión de Investigación del Hospital Universitario La Paz de Madrid and Comisión de Investigación del Hospital Universitario Doce de Octubre de Madrid - approved the protocol and the procedures. All participants provided written informed consent.

Data collection

Socio-demographical data, use of alcohol/illicit drugs, medical history, adherence determined by self-reported missed doses in the last 30 days and use of prescribed medications were obtained by self-report questionnaires, interview or clinical and laboratory records.

Fasting blood plasma samples were collected. Levels of glucose, cholesterol (total, low-, and high-density lipoprotein), triglycerides and insulin were measured using standard methods in the sites' certified clinical laboratories. Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) formula: (insulin in mU/ml x glucose in mmol/L)/22.5. Current CD4 cell count and HIV viral load were determined, respectively, using

flow citometry and automatized RNA extraction in an AmpliPrep instrument (Roche Diagnostics, Mannheim, Germany) followed by quantification using the COBAS AMPLICOR MONITOR HIV-1 test version 1.5 (Roche Diagnostic Systems, Branchburg, NJ).

Comorbidities were classified in three categories: medical comorbidities (hypertension, dyslipidaemia, diabetes mellitus, ischemic heart disease, heart failure, chronic renal failure, thyroid disorders and peripheral arterial disease); neurological comorbidities (history of CNS infection, stroke, cerebral trauma and epilepsy) and psychiatric comorbidities (history of past mood disorders and current or past anxiety disorders). We categorized HCV as no infection, past infection (spontaneous viral clearance or successfully treated) and active infection (detectable HCV plasma viremia).

Psychopathological screening and neuropsychological assessment

All patients completed the depression subscale of the Hospital Anxiety and Depression Scale (HADS-D) at the screening visit. Patients were interviewed by an experienced psychologist in conducting structured interviews if their HADS-D score was equal or above 8. Patients who fulfilled criteria for current major depression (DSM-IV criteria) were excluded and derived to the Psychiatric Service. They could be subsequently enrolled if achieved clinical remission after six months of antidepressant treatment.

The neuropsychological battery included 14 neuropsychological measures that estimate the functioning in seven cognitive and motor domains: attention span/working memory, mental flexibility, verbal fluency, processing of information speed, learning, delayed recall, and fine motor skills. The test battery was created following the 2007 ANN consensus (Antinori et al., 2007). The structure of the test battery was based on the CHARTER study, a large cohort in which HIV+ patients have ongoing monitoring of neuropsychological functioning (Heaton et al., 2010). To estimate and control for premorbid cognitive functioning, the Wechsler Adult

Intelligence Scale (WAIS-III) Vocabulary subtest was included. Table 2 describes the neuropsychological measures used in the present analysis.

The best available normative data for our sample at the moment of the correction were used to covert 14 raw test scores to 14 normalized Z-scores, which were further converted into left-truncated T-scores ranging from 0 to 100. T-scores are normally distributed and have a mean of 50 and standard deviation (SD) of 10 in normal population. Normative data were extracted from different normative studies for individual tests. Due to the larger sample sizes in the moment of the test correction, we applied normative data extracted from US population in most of the neuropsychological measures. We only had suitable normative data from Spanish population for the Trail Making Test forms A and B and the Verbal Selective Reminding Test (VSRT) of verbal memory. Verbal memory measures were age–education–sex corrected and Trail Making Test measures age–education corrected. For each neuropsychological measure, we categorized deficits as mild, if the normalized Z-score was equal or below 1SD and above 2SD below the mean from the reference population, or moderate if equal or below 2 SD (Table 2).

Table 2. Neuropsychological measures and normative sample

NEUROPSCHOLOGICAL DOMAINS AND TESTS	NEUROPSYCHOLOGICAL MEASURES AND DESCRIPTION	NORMATIVE STUDIES
Attention span/Working memory		
WAIS-III Digit Span	*Number of correct responses (sum Forward and Backward measures).* The examiner read series of numbers (eg. 1,2,5) and participants have to immediately repeat them, in same (forward) and inverse order (backward).	n=2,450 (aged 16-89). US population (Wechsler, 1997)
Mental flexibility		
Stroop Colour and Word Test	*Interference score (IS).* In the first trial (W) participants read colour words printed in black; in the second trial (C) they read the colour tune of XXX symbols and; in the third trial (CW) they read the colour tune ignoring the word written (eg. GREEN writes in red colour). They read as much items as they can in 45 seconds. *IS= WC- (W x C/W+C)*	n=300 (aged 15-90) (Golden & Freshwater, 2002)
Trail making Test form B	*Time in second.* Patients draw lines connecting alternatively numbers and letters (eg.1-A-2-B…). They should be performed as quickly and accurately as possible.	n=223 (aged 16-80). Spanish population (Periáñez et al., 2007)
Verbal fluency		
Controlled Oral Word Association Test (FAS)	*Number of correct words in 60 seconds.* Participants say as many words as possible in 1 minute for each of the letters F, A, and S. The total score include the sum of words per each trial (F+A+S)	N=1300 (aged 16-95). US population (Tombaugh, Kozak, & Rees, 1999)
Category Fluency (Animals)	*Number of correct words in 60 seconds.* Participants say as many animals as possible in 1 minute.	N=1300 (aged 16-95). US population (Tombaugh et al., 1999)
Speed of information processing		
* Trail making Test- Form A	*Time in seconds.* Patients draw lines sequentially connecting in ascending order 25 encircles numbers distributed on a sheet paper (1-2-3-4…). They should perform as quickly and accurately as possible.	n=223 (aged 16-80) Spanish population (Periáñez et al., 2007)
WAIS-III Digit Symbol	*Number of correct symbol minus errors (limit: 120 seconds).* The participants copy, as fast as possible, under a series of digits a corresponding symbol. Each symbol corresponds to a number and correspondences are showed above.	n=2,450 (aged 16-89). US population (Wechsler, 1997)
WAIS-III Symbol Search	*Number of correct items minus errors (limit: 120 seconds).* The participants decide whether at least one of two symbols is repeated in an adjacent series of five symbols.	n=2,450 (aged 16-89). US population (Wechsler, 1997)

Table continued on the next page

Continued from table 2

Learning		
Verbal Selective Reminding Test	*Consistent Long-Term Retrieval score (words remembered at least in the last two trials):* represents words that entered in the long-term store, during 6 learning trials. The examiner read the same 12 words for each trial.	n=884 (aged 15-93). Spanish population (Morales et al., 2010)
Brief Visuospatial Memory Test-Revised	*Total Learning score (sum of accuracy scores in the 3 learning trials).* Participants draw features and spatial placement of six geometric figures learned during 10 second per trial. Each trial score is calculated depending on accuracy in form and spatial location.	n=588 (aged 18-79). US population (Benedict, 1997)
Delayed recall		
Verbal Selective Reminding Test	*Delayed Recall score (number of words remembered).* After 30 minutes patients remember as many words as they can from the list presented in the prior learning trials.	n=884 (aged 15-93). Spanish population (Morales et al., 2010)
Brief Visuospatial Memory Test– Revised	*Delayed Recall score (accuracy score).* Participants draw the features and spatial placement of each one of six geometric figures learned during the learning trials.	n=588 (aged 18-79). US population (Benedict, 1997)
Fine motor skills		
Grooved Pegboard Test-Dominant hand	*Time in seconds.* Participants placed pegs into slots differently positioned with their dominant hand. They should perform as quickly and accurately as possible.	1616 (aged 10-60). US population (Trites, 1977)
Grooved Pegboard Test- Non dominant hand	*Time in seconds.* Participants placed pegs into slots differently positioned with their non-dominant hand. They should perform as quickly and accurately as possible.	1616 (aged 10->60). US population (Trites, 1977)
Premorbid functioning (estimation)		
* WAIS-III Vocabulary subtest	*Total score.* Participants provided word definitions given by the examiner. The score is calculated depending on the accuracy of the answers.	n=2,450 (aged 16-89). US population (Wechsler, 1997)

* Trail making Test form A was included in the Analysis 1 but was not used to estimate global NCI. The Vocabulary subtest did not contribute to the diagnosis of NCI.

Also following the 2007 ANN criteria, global NCI was considered when patients performed at least 1 SD below the mean on the normative data in at least two ability domains (Antinori et al., 2007). Raw scores of each neuropsychological measure were converted to demographically corrected standard Z scores, by a computerized application. To estimate the domain ability, a Z score was calculated as the mean of the two neuropsychological measures that compose the domain.

Current use of tobacco, cannabis, cocaine, heroin, methadone and designer drugs was explored by interview. Sociodemographical data, antiretroviral adherence level, and psychiatric comorbidities (history of past mood disorders and current or past anxiety disorders) were also recorded during the neuropsychological assessment visit.

Statistical methods

Sample characteristics were described using absolute and relative frequencies for categorical variables and means (SD) or medians (IQR) for continuous variables. Chi-squared test and Student's t or the non-parametric Mann–Whitney U test were used, respectively, to compare baseline characteristics.

To determine differences in the neuropsychological pattern of functioning among our HIV+ patients, T-scores were described using the mean (SD) and compared using Student's t between the triple therapy and the monotherapy group, for each of the 14 neuropsychological measures. Effect sizes were calculated to determine the magnitude of any differences in performance using Cohen's d (Howell, 2002). The effect size was classify as small if it was between 0.2 and 0.5, medium if between 0.5 and 0.8, and large if over 0.8 (Cohen, 1988). To determine the independent effect of treatment group over each neuropsychological measure, multivariate linear regressions were conducted with an estimative approach, retaining in each model

confounder variables that produced a change greater than 15 % in the regression coefficient of interest. We evaluated the following as potential confounders: age, sex, ethnicity, mode of HIV transmission, years on ART, years with suppressed HIV viremia, prior single blip, current CD4 count, nadir CD4 count, years of education, current use of non- prescribed drugs, presence of medical, neurological and psychiatric comorbidities, coinfection with HCV, use of statin, triglycerides, total cholesterol/HDL ratio, HOMA-IR index, and WAIS-III Vocabulary subtest score. Finally, proportion of patients with mild neurocognitive deficits (equal or below -1 SD, equivalent to $T < 40$) and proportion of patients with moderate deficits (equal or below -2 SD, equivalent to $T \leq 30$) were described in the whole sample and by group of treatment using proportions. Proportions of mild and moderate deficits were compared between protease inhibitor monotherapy and triple therapy group using Chi-squared or Fisher's exact test when expected number of cases in one cell of the contingency table was below 5.

The same unadjusted and adjusted analyses were conducted to explore differences in the neuropsychological pattern only in patients with global NCI. Due to the smaller sample size, multivariate regression models were built by a priori selection of the maximum number of confounders allowed in the models. Selected confounders were as follows: age, ethnicity, years of education, WAIS-III Vocabulary subtest score, and coinfection with HCV. Also, proportion of patients with mild and moderate deficits was compared between antiretroviral strategies in the neurocognitively impaired subgroup of patients.

Significance level was established at $p < 0.05$, and all analyses were conducted using Stata software (V.11.1, Stata Corporation, College Station, TX, USA).

4.1.2.Results

Patient's characteristics

A total of 191 participants were included in the analysis, 95 in the triple therapy group (48 %) and 96 in the protease inhibitor monotherapy (52%). Study patients were predominantly male, Caucasian, highly adherent to antiretroviral medication, infected through sexual route, and with good current immune status. Almost half of the sample had either past or active hepatitis C virus infection (Table 3).

Compared to patients receiving protease inhibitor as part of triple therapy regimens, patients on monotherapy were more frequently Caucasian, slightly older, had received more prolonged ART, had higher current CD4 count, were known to have HIV- infection for a longer time, had a more prolonged period with undetectable viral load, and also had higher frequency of medical disease history. Darunavir was significantly more frequently used than lopinavir in the monotherapy group. The metabolic profile was worse in the monotherapy group with higher triglycerides, higher total cholesterol/HDL, and also higher frequency of statin treatment. We found no significant differences in other variables such as years of education, sex, history of neurological and psychiatric comorbidities, hepatitis C virus infection, or frequency of AIDS (Table 3).

Table 3. Patient´s characteristics

	TT	MT	Total	
	N= 95	N=96	N=191	P values
Current protease inhibitor. N (%)				
Darunavir/ritonavir	25 (26.3)	43 (44.8)	68 (35.6)	<0.01
Lopinavir/ritonavir	70 (73.7)	53 (55.2)	123 (64.4)	
Adherence level <100%. N (%)	25 (26.3)	18 (18.8)	43 (22.5)	0.23
Male. N (%)	70 (73.7)	70 (72.9)	140 (73.3)	0.91
Mode of HIV transmission. N (%)				
Men who have sex with men	29 (30.5)	30 (31.3)	59 (30.9)	0.85
Heterosexual	30 (31.6)	25 (26.0)	55 (28.8)	
Intravenous drug use	30 (31.6)	34 (35.4)	64 (33.5)	
Other	6 (6.3)	7 (7.3)	13 (6.8)	
Ethnicity. N(%)				
Caucasian	79 (83.2)	92 (95.8)	171 (89.5)	<0.01
Other	16 (16.8)	4 (4.2)	20 (10.5)	
Age. Median (IQR)	44.7 (40.6-48.4)	47.4 (44.8-51.4)	46.4 (42.3-49.6)	<0.01
Years of education. Mean (SD)	11.3 (4.1)	10.4 (4.4)	10.8 (4.3)	0.13
WAIS-III Vocabulary subtest. Mean (SD)	0.3 (0-1)	0.7 (0-1)	0.3 (0-1)	0.64
Use of non-prescribed drugs. N(%)				
Never	43 (46.7)	50 (52.1)	93 (49.5)	0.66
Past	25 (27.2)	26 (27.1)	51 (27.1)	
Active	24 (26.1)	20 (20.8)	44 (23.4)	
AIDS. N(%)	60 (63.2)	59 (62.1)	119 (62.6)	0.88
Years with HIV. Median (IQR)	15.1 (7.2-19.9)	17.2 (12.7-21.5)	15.8 (11.0-20.7)	<0.01
Years virologically suppressed. Median (IQR)	4.8 (2.9-8.9)	7.5 (4.5-10.0)	6.6 (3.7-9.2)	<0.01
Prior blip. N (%)	20(21.1)	14(14.6)	34(17.8)	0.17
Years on antiretroviral therapy. Median (IQR)				
Total	10.7 (4.8-15.7)	14.1 (10.7-15.9)	12.8 (7.1-15.8)	<0.01
TT	10.7 (4.8-15.7)	10.7 (7.0-13.7)	10.7 (5.7-14.5)	0.80
MT	-	2.3 (1.7-3.2)	-	-
CD4 nadir (cell/mm^3). Median (IQR)	153 (49-255)	182 (76-288)	177 (54-270)	0.11
Current CD4 (cells/mm^3). Median (IQR)	560 (440-754)	629.5 (476-845.5)	597 (450-818)	<0.05
Neurological disease history. N(%)	12 (12.6)	10 (10.4)	22 (11.5)	0.63
Psychiatric disease history. N (%)	19 (20.0)	24 (25.0)	43 (22.5)	0.44
Medical disease history*. N (%)	35 (36.8)	49 (51.0)	84 (44.0)	0.05
Statin treatment. N(%)	14 (15.9)	26 (28.0)	40 (22.1)	0.05
HADS-D. Median (IQR)	2 (0-5)	2 (1-4)	2 (1-4)	0.73
Triglycerides (mg/dl). Median (IQR)	136.5 (108-197)	183 (128-233)	164 (115-225)	<0.01
Total Cholesterol/HDL ratio . Median (IQR)	3.9 (3.3-4.7)	4.5 (3.6-5.6)	4.2 (3.4-5.3)	0.02
HOMA index. Median (IQR)	1.8 (1.1-2.7)	2.2 (1.4-3.6)	2.0 (1.3-3.3)	0.06
Hepatitis C infection. N (%)				
No	48 (52.8)	52 (54.7)	100 (53.8)	0.54
Past	19 (20.9)	24 (25.3)	43 (23.1)	
Active	24 (26.4)	19 (20.0)	43 (23.1)	

TT=Triple therapy. MT= Monotherapy. *Medical disease: hypertension, dyslipidaemia, diabetes mellitus, ischemic heart disease, heart insufficiency, chronic renal failure, thyroid disorders and peripheral arterial disease. HADS-D=Hospital Anxiety and Depression Scale- Depression subscale. HDL= High Density Lipoproteins. HOMA= Homeostasis Model Assessment.

Neuropsychological test results in the sample of patients receiving protease inhibitor monotherapy versus triple therapy

Regarding average performance in each neuropsychological measure, the unadjusted analysis showed no significant differences between treatment groups. However, patients on protease inhibitor monotherapy tended to perform slightly better on digit span, verbal learning and verbal recall measures, with p values between 0.06 and 0.08. All differences between groups had small effect sizes ($d < 0.50$). In the adjusted analysis, three neuropsychological measures significantly differed by treatment group. Verbal learning and verbal delayed recall scores were lower in the triple therapy group, while patients on monotherapy had worse scores in fine motor skills with dominant hand (Table 4).

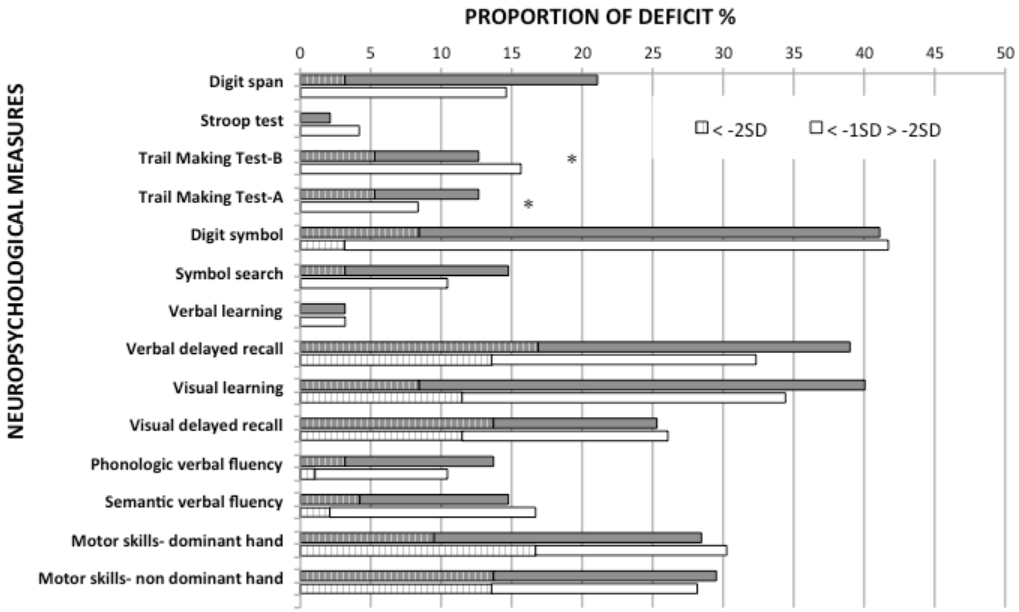
When we analysed rates of alterations in each neuropsychological measure, we did not find differences in the proportion of patients with mild neurocognitive/motor deficits by treatment group. However, moderate deficits were significantly more frequent in patients on triple therapy for the Trail Making Test form A (protease inhibitor monotherapy 0 %, triple therapy 5.3 %; p=0.03) and form B (protease inhibitor monotherapy 0 %, triple therapy 5.3 %; p=0.03). None of remaining neuropsychological measures showed significant differences in proportion of moderate deficits between two treatment groups (Figure 5).

Table 4. Comparison of test results for patients on monotherapy vs. triple therapy

NEUROPSYCHOLOGICAL MEASURES	T Score Mean (SD)			Effect Size (Cohen's d)	p-value (crude) *	Regression Coefficient	p-value (adjusted)*
	TOTAL (n=191)	TT (n=96)	MT (n=95)				
Digit span	51.2 (9.1)	50.0 (9.5)	52.4 (8.6)	0.26	0.08	1.75	0.10
Stroop test	53.7 (7.3)	53.5 (7.0)	53.8 (7.5)	0.04	0.78	-0.10	0.94
Trail Making test form B	48.8 (9.3)	48.8 (10.6)	48.7 (8.0)	0.00	0.98	-1.16	0.44
Trail Making test form A	53.4 (11)	53.0 (11.9)	53.8 (10.0)	0.07	0.64	-1.32	0.46
Digit symbol	43.1 (7.2)	42.3 (7.6)	43.9 (6.8)	0.21	0.14	-.81	0.37
Symbol search	50.8 (8.6)	50.0 (8.8)	51.5 (8.3)	0.18	0.22	-1.19	0.29
Verbal learning	55.6 (9.6)	54.3 (8.7)	56.9 (10.5)	0.28	0.06	3.51	0.02
Visual learning	43.7 (13)	42.4 (13.5)	44.9 (12.5)	0.19	0.18	2.33	0.24
Verbal delayed recall	44.8 (11.3)	43.3 (10.6)	46.2 (11.9)	0.25	0.08	4.91	<0.01
Visual delayed recall	47.8 (113.5)	47.0 (14.1)	48.5 (12.9)	0.12	0.42	0.50	0.81
Phonologic verbal fluency	52.7 (11.2)	51.4 (10.7)	54.0 (11.7)	0.24	0.10	2.13	0.21
Semantic verbal fluency	52.4 (12.6)	52.3 (12.5)	52.5 (12.6)	0.01	0.92	-0.76	0.70
Motor skills dominant hand	45.7 (13.9)	47.2 (13.7)	44.1 (14.0)	0.23	0.12	-4.68	0.02
Motor skills non dominant hand	43.5 (12.4)	43.8 (11.4)	43.2 (13.3)	0.10	0.52	-0.53	0.80

Total= the whole sample of HIV+ patients (n=191). TT=Triple therapy. MT= Monotherapy. SD = Standard Deviation. T score for each measure is the standardization from raw scores. Standarized T-scores have a normal distribution with a mean of 50 and a standard desviation of 10. Verbal learning was adjusted by years on ART and triglycerides; visual learning by vocabulary subtest, age, time virologically suppressed, HOMA index, type of protease inhibitor prescribed, ethnicity, designer drug use, triglycerides, sex, prior single blip, alcohol use and years of education; verbal delayed recall by years on antiretroviral treatment, marihuana use, triglycerides, type of protease inhibitor prescribed and vocabulary subtest; and motor skill with dominant hand by vocabulary subtest, nadir CD4 count and HOMA index.

Figure 5. Proportion of patients with mild and moderate deficits in the neuropsychological measures by treatment regimen



Grey and white bars indicate patients receiving triple therapy or monotherapy, respectively. Striped sections of the bars indicate proportions of patients with moderate deficits: equal or lower than 2 standard deviations below mean of the reference population. Non-striped sections of the bars indicate proportion of patients with mild deficit: a result between 1 and 2 standard deviation below mean of the reference population. Asterisk indicates differences statistically significant (p<.05). Significant differences found only in the proportion of patients with moderate deficits in Trail Making Test form A and B.

Neuropsychological test results in neurocognitively impaired patients receiving protease inhibitor monotherapy versus triple therapy

Prevalence of global NCI was 31.6 % (n= 30) in patients receiving triple therapy regimens and 22.9 % (n= 22) in patients on protease inhibitor monotherapy.

Unadjusted analysis revealed significant worse average performance in the verbal delayed recall measure among patients in triple therapy but worse outcomes in motor skills with dominant hand among patients receiving monotherapy (Table 5). Verbal delayed recall (d= 0.63) and motor skills with dominant hand (d= 0.54) had medium effect size in magnitude of

differences while all the remaining neuropsychological measures had small effect size (d< 0.5). Adjusted analysis confirmed the significant differences by treatment group in both neuropsychological measures and revealed a trend toward significant values in verbal learning (p= 0.06) with poorer performance in patients receiving triple therapy (Table 5).

Table 5. Comparison of test results for patients on monotherapy vs. triple therapy in patients with neurocognitive impairment

NEUROPSYCHOLO-GICAL MEASURES	T Score Mean (SD)			Effect Size (Cohen's d)	p-value (crude)	Regression Coefficient	p-value (adjusted)
	TOTAL	TT	MT				
Verbal learning	51.6 (8.2)	49.7(7.9)	53.5(8.5)	0.47	0.10	5.09	0.06
Verbal delayed recall	41.1 (10.3)	37.9(8.6)	44.3(12.0)	0.63	0.03	6.11	0.05
Motor skills dominant hand	37.4 (13.5)	41.1(14.4)	33.7(12.5)	0.54	0.06	-7.58	0.05

TT=Triple therapy. MT= Monotherapy. SD = Standard Deviation. Standardized T-scores have a normal distribution with a mean of 50 and a standard deviation of 10. Table included the NP measures that revealed differences by treatment group after adjustment. Selected confounders for adjustments were: age, ethnicity, years of education, Vocabulary subtest and co infection with hepatitis C virus.

We also found a higher proportion of mild deficit in the Trail Making Test form A in the triple therapy group compared to the monotherapy group (36.7 vs. 13.6 %; p = 0.03). However, patients receiving protease inhibitor monotherapy had higher proportion of mild deficits in the measurement of motor skills with dominant hand (73.7 vs. 43.3 %; p = 0.03). Regarding moderate dysfunctions, we did not find a higher proportion of deficits in the monotherapy group, in any of the neuropsychological measures. Trail Making Test forms A and B showed a trend towards a higher proportion of moderate deficits in patients receiving triple therapy (16.7 vs. 0.0 %; p=0.06).

Neuropsychological test results in the whole sample of aviremic HIV+ patients

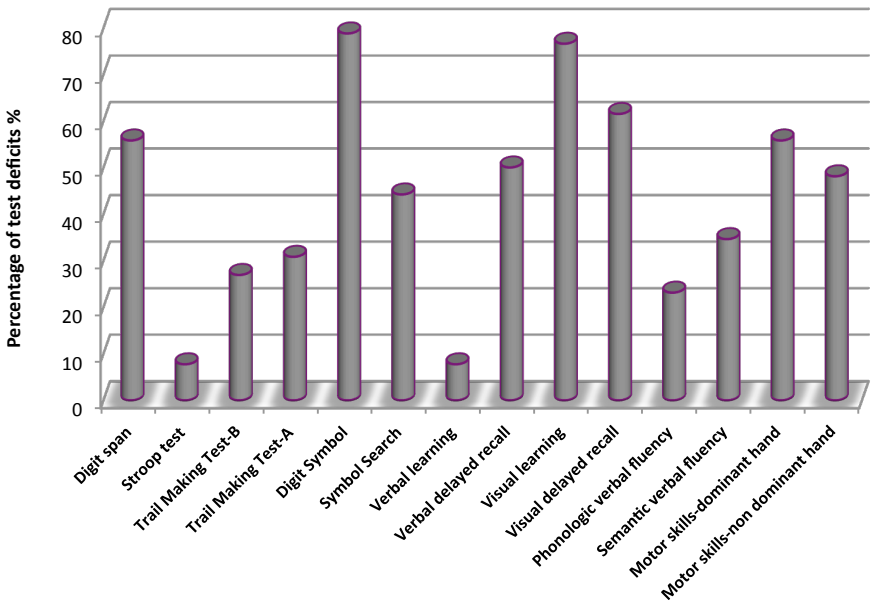
Prevalence of global NCI was 27.3% (n= 52) in our whole sample of aviremic patients (n=191),

regardless of the type of ART received. As a group, HIV+ patients had not large deviations from the expected neuropsychological values in the normative data. Average values in most of the test applied were closer to the population mean (T=50) (Table 4).

As shows Figure 5, the highest proportion of deficits were obtained in the same neuropsychological measures, regardless of the type of treatment. In the whole HIV+ sample, over 20% of patients had deficits in the following measures: digit symbol, visual learning, verbal delayed recall, fine motor skills, visual delayed recall and digit span.

Over 20% of patients with global NCI (n=52) had deficits in most of the neuropsychological measures. However, more than 50% of patients had deficit in the following measures: digit symbol, visual learning, visual delayed recall, fine motor skills, verbal delayed recall and digit span (Figure 6).

Figure 6. Proportion of neurocognitively impaired patients with deficits in each neuropsychological measure



4.2. Analysis 2. Facial emotion processing deficits among long-term aviremic HIV-patients and effect of receiving boosted protease inhibitor monotherapy

4.2.1.Methods

Participants

We included a total of 107 HIV+ participants and 40 healthy adults. HIV+ participants were recruited from those HIV+ patients who completed the one-year follow-up visit in the Picasso Study (Pérez-Valero et al., 2014b). Of the 134 HIV+ patients who participated in the follow-up standard neuropsychological assessment, a total of 107 completed the facial emotion processing protocol of tasks. The remaining 27 patients did not complete the protocol because of time constraints. The rest of procedures were the same as at baseline described in the Analysis 1.

To participate in the follow-up visit of the Picasso Study patients had to remain receiving ART and plasma virologically suppressed - less than 50 HIV RNA copies/millilitres- for at least two years. Healthy adults were relatives of patients recruited from a Spanish Neurology Service and professionals from the recruitment center. We matched HIV+ and healthy adults participants for gender and age.

Standard neuropsychological assessment and medical measures

Comprehensive neuropsychological assessment and laboratory measures were the same as in the Analysis 1. Briefly, the test battery included 14 measures of attention and working memory, mental flexibility, verbal fluency, verbal and visual learning, verbal and visual delayed recall, speed of information processing and fine motor skills, commonly altered in HIV+ patients (Table 2). Following the 2007 ANN criteria, patients were classified as neurocognitively

impaired or non-impaired. The psychologist recorded history of substance abuse and relevant sociodemographical data.

All HIV+ participants performed a standard venipuncture the same week of the neuropsychological assessment, and plasma HIV RNA level and plasma CD4 counts were obtained. A physician registered medical variables. Time since HIV diagnosis, time virologically suppressed, time on ART, current antiretroviral regimen, AIDS status, mode of HIV transmission, prior treatment with peginterferon and current HCV status were captured from a computerized database.

Facial emotional processing tasks

All HIV+ participants and healthy adults performed a computerized battery of tests comprised of six tasks that required discrimination, memorization and recognition of basic facial emotions. The tasks were adapted from five subsets of the Florida Affect Battery (FAB), which has been used in several clinical studies (Bowers, Blonder, & Heilman, 1991; Bucks & Radford, 2004; Carvajal, Rubio, Martín, Serrano, & García-Sola, 2009; Milders, Ietswaart, & Crawford, 2008). Each task consists of ten items that are presented to all participants in the same order. Figure 7 shows an item of each task. Tasks are grouped in the following blocks:

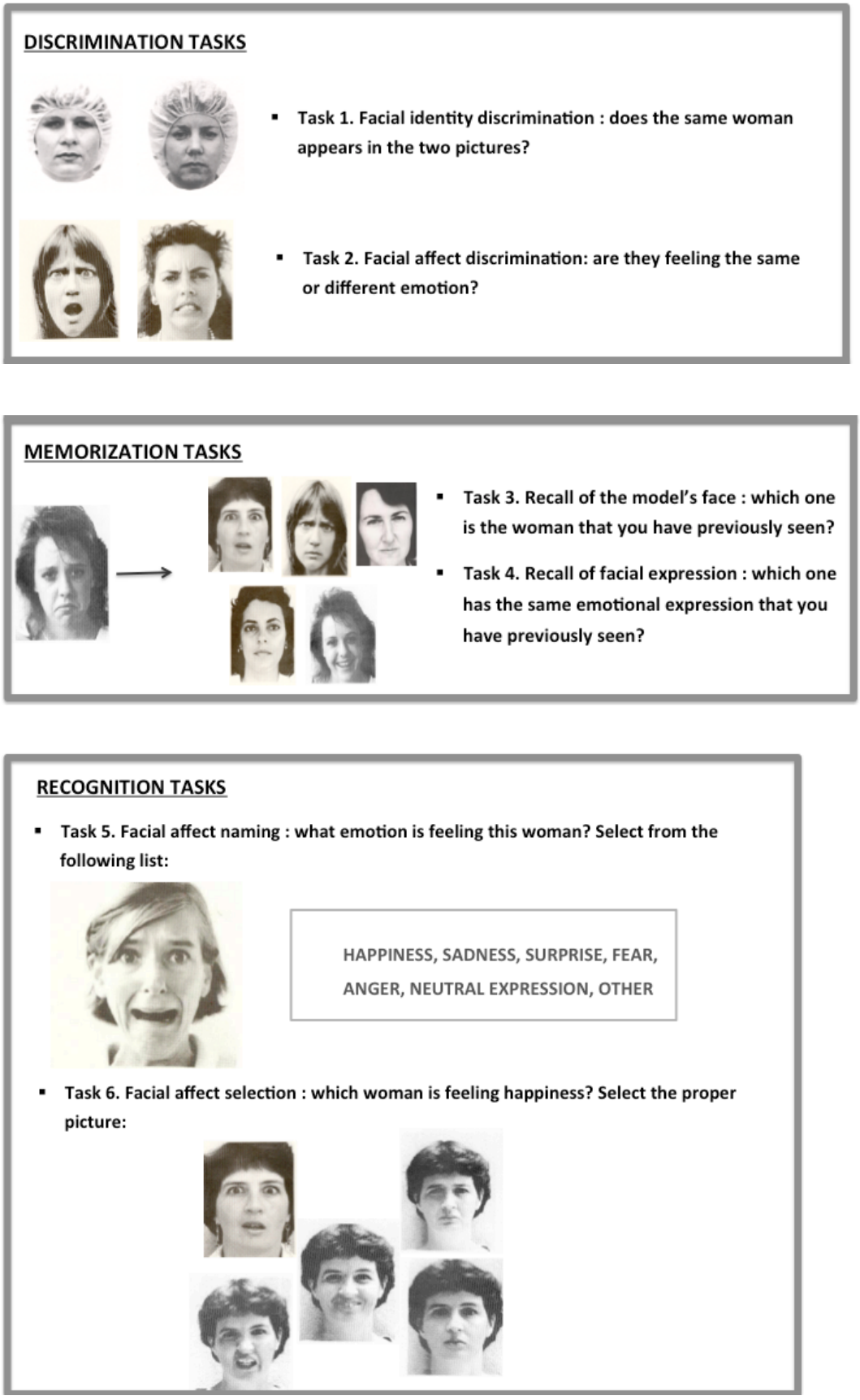
Block 1 included two discrimination tasks. Slides in the first task -facial identity discrimination- showed simultaneously two photographs of neutral facial expressions. Participants had to decide whether the photographs correspond to the same or different women. Both pictures were of the same woman in half of slides and corresponded to two different women in the other half. Slides in the second task -facial affect discrimination- showed two photographs of different women. Participants had to answer whether the two faces depict the same or different emotional expressions. Both women displayed the same emotional expression in half

of the slides. The first task requires discriminating the identity of the women and the second task requires discriminating the emotional expressions.

Block 2 included two memorization tasks. The memory tasks were adapted from the FAB facial affect matching task and used on a previous study (Carvajal et al., 2009). Both tasks showed for five second the slide of a woman who expresses a facial emotional expression (neutral, happiness, sadness, anger and fear). The slide is then replaced by other slide that showed the pictures of five women with different facial expressions. Participants were exposed to the same stimuli in both tasks. In the third task -recall of the model's face-, subjects had to identify the woman seen before, regardless of her facial expression. In the fourth task -recall of facial expression- participants had to identify the emotional expression seen before, regardless of the identity of the model.

Block 3 included two recognition tasks. In the fifth task -facial affect naming-, participants named the emotion corresponded to each face individually presented selecting from five facial emotions (neutral, happiness, sadness, anger and fear). The sixth task -facial affect selection- showed slides displaying simultaneously five photographs of the same model, each of which expressed different facial emotions. The examiner said verbally an emotion (neutral, happiness, sadness, anger and fear) and participants should select the photography that corresponds with that emotion. Thus, participants did not have to verbally name.

Figure 7. Item examples of each facial processing task



Data analysis

Absolute and relative frequencies for categorical variables and median (IQR) for continuous variables were used to describe sample characteristics.

To compare HIV+ and healthy adult participants in overall facial processing, average raw scores by group and block of tasks (discrimination, memory and recognition) were calculated using a two factors group task interaction effect with task as repeated factors (analysis of variance, ANOVA). Raw scores were calculated as the number of correct response in each task.

The nonparametric Mann-Whitney U-test was conducted to determine whether HIV+ patients had worse accuracy in recognition of each basic facial emotion (neutral, happiness, sadness, anger and fear) compared to the healthy adults group.

To explore the ability of medical factors and neurocognitive status to predict poorer outcomes in the HIV+ sample, multiple logistic regressions were conducted in a stepwise mode in those measures in which the HIV+ group had a poorer performance. Dependent variables were dichotomized using all correct answer or at least one error. Nine covariates were forced to enter in each regression analysis: current CD4 count (0-350; over 350), nadir CD4 count (under 200; over 200), years on ART, years with suppressed HIV viremia, years since HIV diagnosis, hepatitis C serostatus (active; no active), peginterferon use (prior exposed; not prior exposed), presence of global NCI (yes; no) and the WAIS-III Vocabulary subtest scores.

To separately explore the impact of NCI, HCV or type of ART on overall performance in each facial processing tasks, we compared average raw scores yielded by T-test for independent samples between HIV+ patients: with global NCI vs. without NCI, with current active HCV vs. non-active HCV, and receiving protease inhibitor in monotherapy vs. protease inhibitor as part of triple therapy regimens. Effect sizes were calculated by Cohen´s d.

Significance level was established at p< .05, and all analyses were conducted using IBM® SPSS® Statistics 20.

4.2.2. Results

Patient's characteristics

The majority of HIV+ participants (n=107) were male, middle-aged, Caucasians, who had been infected through sexual intercourse and with good current immune status. Most of the HIV+ sample did not have active HCV infection and less than a quarter had been previously treated with interferon. Ninety-three HIV+ participants (87%) were receiving the same antiretroviral regimen - protease inhibitor in monotherapy or as part of triple therapy- for at least two years, while 14 patients (13%) had switched the antiretroviral regimen during the last year due to toxicity, simplification, reintensification or enrolment in clinical trials (see Table 6). The healthy adults group (n=40) included predominantly middle-aged males (65%). We did not find significant differences between healthy adults and HIV+ participants in average age (t (145)= 0,96, p= .34) or gender proportion (χ^2 (1, N=147) = 2,28, p= .13).

Table 6. Baseline characteristics of HIV+ participants (n=107)

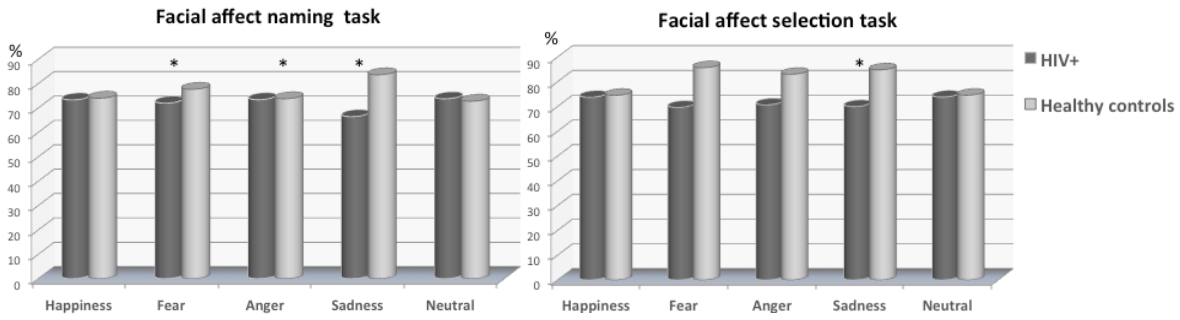
Neurocognitive impairment. N (%)	26 (24.3)	Years since HIV diagnosis. Median (IQR)	16.7 (11.6-22.5)
Age. Median (IQR)	47.4 (43-52)	Years on antiretroviral therapy. Median (IQR)	13.7 (7.4-16.8)
Years of education. Median (IQR)	10 (8-14)	Years virologically suppressed. Median (IQR)	6.6 (4.5-10.2)
Caucasian. N (%)	95 (88.8)	Mode of HIV transmission. N (%)	
AIDS diagnosis. N (%)	71 (66.4)	Men who have sex with men	34 (31.8)
Current antiretroviral regimen. N (%)		Heterosexual	32 (29.9)
Triple therapy	51 (47.6)	Intravenous drug use	31 (29.0)
Monotherapy	42 (39.3)	Other	10 (9.3)
Switches during the prior year	14 (13.1)	Hepatitis C co-infection. N (%)	
Prior treatment with interferon. N (%)	22 (20.6)	No	58 (54.2)
Current CD4 (cell/mm3). Median (IQR)	595.5 (421-711.8)	Past	24 (22.2)
Nadir CD4 (cell/mm3). Median (IQR)	170 (50-272)	Active	25 (23.4)

Facial emotion recognition in HIV+ patients

Overall performance in the facial emotion recognition tasks did not differ between HIV+ participants and a group of healthy adults. Repeated-measures ANOVA did not show a significant effect of group or interactions between tasks and HIV-infection status. We only found a main significant effect for type of task in the block of recognition. Both healthy adults and HIV+ participants had a better performance in the facial affect selection task than in the facial affect naming task ($F(1,145) = 112.60$, $p < .0001$). The selection task that does not require verbal labelling of emotions was easier for both groups. Moreover, the effect size of differences between groups was small in both tasks: the facial affect naming ($d = .24$) and the facial affect selection ($d = .05$).

We also compared the distribution of correct response between HIV+ participants and healthy adults in the recognition of each basic emotion. The HIV+ group had significant lower performance than the healthy adults group on the recognition of several emotions. They had significantly poorer recognition of sadness in the facial affect naming task ($z = -2.43$, $p = .015$) and poorer recognition of sadness ($z = -2.52$, $p = .012$), anger ($z = -2.09$, $p = .036$) and fear ($z = -2.30$, $p = .021$) in the facial affect selection task (Figure 6).

Figure 8. Percentage of correct response in each recognition task by group



* Significant differences ($p < .05$) in distribution of correct response calculated by Mann-Whitney U-test. Axis Y= % of correct responses

Facial discrimination and memorization in HIV+ patients

We did not find differences between HIV+ participants and a group of healthy adults in overall performance of any facial discrimination or memorization tasks. No significant effect of group or interactions between task and group were revealed in any of the two repeated-measures ANOVA conducted. Discrimination and memory blocks only had a main effect for type of task. Healthy adults and HIV+ participants performed better the facial discrimination task than the facial affect discrimination task ($F(1,145) = 107.18$, $p < .0001$). Also, the recall of the model's face task was easier than the recall of facial expressions task for both groups ($F(1,145) = 120.23$, $p < .0001$). Tasks that do not require explicit emotional processing were performed better in either discrimination or memory blocks. Effect size of differences between groups was small in all the tasks: facial discrimination ($d = .02$), facial affect discrimination ($d = .22$), recall of the model's face ($d = .05$) and recall of facial expression ($d = .11$).

Facial emotional processing impairment among HIV+ participants and effect of medical factors and neurocognitive status

Multiple logistic regressions were conducted to identify whether medical variables or NCI status predicted poorer performance in those variables in which HIV+ participants had significant lower accuracy than healthy adults: recognition accuracy of sadness in the facial affect naming task and, recognition accuracy of sadness, anger and fear in the facial affect selection task.

Medical factors, NCI status or the WAIS-III Vocabulary subtest scores were not significantly associated with a lower recognition of sadness in the facial affect naming task ($\beta = -0.64$, Wald χ^2 (1, N=107)= 2.50, Exp (β)=0.52; 95% CI [0.24 –1.27], p= .12).

However, the Vocabulary subtest scores were independently associated to the recognition accuracy of sadness (β = 0.72, Wald χ^2 (1, N=107)= 6.49, Exp (β)=2.06; 95% CI [1.18 –3.58], p= .011) and anger (β=0.69, Wald χ^2 (1, N=107)= 5.94, Exp (β)=1.99; 95% CI [1.14 –3.45], p= .015) in the facial affect selection task. Medical factors or global NCI status did not predict lower recognition accuracy. HIV+ participants with lower Vocabulary scores had poorer recognition of sadness and anger in the recognition task that does not require verbal labelling (the affect naming task).

Co-infection with HCV showed a trend towards significance as a predictor of lower recognition of fear in the facial affect selection task (B= -0.92, Ward χ^2 (1, N=107)= 3.78, Exp (β)=0.40; 95% CI [0.016 –1.007], p= .052).

Impact of global neurocognitive status, type of antiretroviral regimen and active HCV infection on overall facial processing

In the analyses conducted to compare the average raw scores depending on the global cognitive status, HIV+ participants with NCI had lower performance than those without NCI in both memorization tasks. Moreover, there was a trend toward poorer performance in patients with NCI in the facial affect naming task (p=.06), although HIV+ patients with NCI did not have a lower performance in the facial affect selection task. We did not find differences in overall facial identity discrimination or facial affect discrimination between patients with and without NCI (Table 7).

Table 7. Facial emotional processing according to neurocognitive status in the HIV+ sample

FACIAL EMOTION PROCESS	Raw Score. Mean (SD)		t value	P value	Cohen´s d
	Neurocognitively non-impaired	Neurocognitively impaired			
Discrimination					
Facial discrimination	9.72 (0.52)	9.65 (0.75)	0.57	0.57	0.01
Facial affect discrimination	8.53 (0.96)	8.28 (1.07)	1.13	0.26	0.25
Recognition					
Facial model naming	7.38 (1.07)	6.88 (1.42)	1.90	0.06	0.40
Facial affect selection	8.86 (1.12)	8.81 (1.20)	0.22	0.83	0.05
Memory					
Recall model´s face*	9.25 (0.93)	8.31 (1.19)	4.18	0.001	0.88
Recall model expression*	7.70 (1.46)	7.38 (1.93)	5.52	0.001	1.48

The effect of different antiretroviral strategies was only tested in HIV+ participants who maintained the same antiretroviral type of regimen for at least two years (n=93). Those who had changed of type of treatment during the previous year were excluded of this analysis. We did not find significant differences between patients receiving protease inhibitor in monotherapy or as part of standard triple therapy in any of the facial processing tasks ($p > .05$). Effect sizes of differences between treatment groups were small in all the tasks ($d < .50$).

HIV+ participants with active HCV compared to those without active HCV-infection had not significant differences of performance or medium/large effect sizes of differences in any of the facial discrimination, memorization or recognition tasks.

4.3. Analysis 3. Emotional prosody recognition in long-term aviremic HIV+ patients and effect of receiving boosted protease inhibitor monotherapy

4.3.1.Methods

Participants

A total of 100 aviremic HIV+ individuals and 46 healthy adults were included in the present analysis. The HIV+ patients performed a newly designed vocal emotion processing test, as part of the standard neuropsychological assessment at the one-year follow-up visit of the Picasso Study (Pérez-Valero et al., 2014b). The rest of procedures were the same as at baseline, previously described in the Analysis 1. All the HIV+ participants consented to perform a standard venepuncture and their medical variables were collected from the clinical history. To participate in the follow-up visit of the Picasso Study, patients had to remain receiving ART and virologically suppressed for at least two years. A subset of thirty-six of these HIV+ patients also completed a 3-Tesla Magnetic Resonance scan as part of the neuro-imaging sub-study (Pérez-Valero et al., 2014a).

Fourty-six healthy adults were matched to the HIV+ participants for age and gender. They were selected from healthy relatives of patients from a Spanish Neurology Service and from healthy volunteers working at the Universidad Autónoma De Madrid and the Hospital Universitario La Paz. In addition, 90 students of the Psychology Degree voluntarily participated in the test design phase.

Standard neuropsychological assessment and laboratory measures

All HIV+ participants underwent a comprehensive neuropsychological test battery to determine presence or absence of NCI. The battery has been described in the Analysis 1 (Table 2). In the

same visit, the psychologist recorded the history of substance abuse and relevant sociodemographical data.

Measures obtained by a standard venepuncture performed the same week that the neuropsychological assessment, and medical variables registered have been detailed in the Analysis 1 (methods section). Briefly, we obtained measures of plasma HIV RNA level, plasma CD4 cell counts, time since HIV diagnosis, duration of virological suppression, time on ART, current antiretroviral regimen, AIDS status, mode of HIV transmission, prior treatments with peginterferon and current HCV serostatus.

Design of the emotional prosody processing test

We designed a vocal emotion (emotional prosody) processing test based on the FAB ninth subtest (Bowers et al., 1991), that consists in matching the vocal emotional prosody to pictures of faces with different emotional expressions.

First, we created five written sentences, similar in words length. Then, we asked to thirty students of Psychology degree to select the emotion contained in each written sentence. The students chose among six written labels provided below each sentence: neutral, happiness, sadness, anger, fear or other. The Spanish written sentences were: “he ganado la medalla de oro en natación” (I´ve won the gold medal in swimming), “ el niño llora sobre la tumba de su madre” (the boy cries over his mother´s grave), “me han pinchado las ruedas del coche” (someone has pinched my car wheels), “la pared del salon es de color blanco” (the wall of the living room is white), and “esta noche creí ver a alguien tras la ventana” (I thought I saw someone in the window last night). All participants (100%) selected the same emotion for each sentence: happiness, sadness, anger, neutral and fear, respectively.

Second, we assigned a vocal emotional prosody to each one of the five phrases (neutral, happiness, sadness, anger or fear) and an actress taped the phrases in a recording studio. Of the total, we chose nineteen phrases and asked to sixty students, different from previous participants, to select one of the five faces from the FAB subtest 4 slide (with neutral, happiness, sadness, anger and fear expressions), depending on the emotional prosody listened and regardless of the verbal content. For the final test we selected sentences in which the level of agreement among participants were higher than 85%. Table 8 shows twelve sentences with agreement over 85% and those items included in the final emotional prosody processing test. We selected two items of neutral, happiness, sadness and fear prosody (one of them emotionally congruent in verbal and emotional prosody content and another incongruent). Only one sentence was included with prosody of anger owing to the low agreement levels. Finally, nine items comprised the new design emotional prosody processing test.

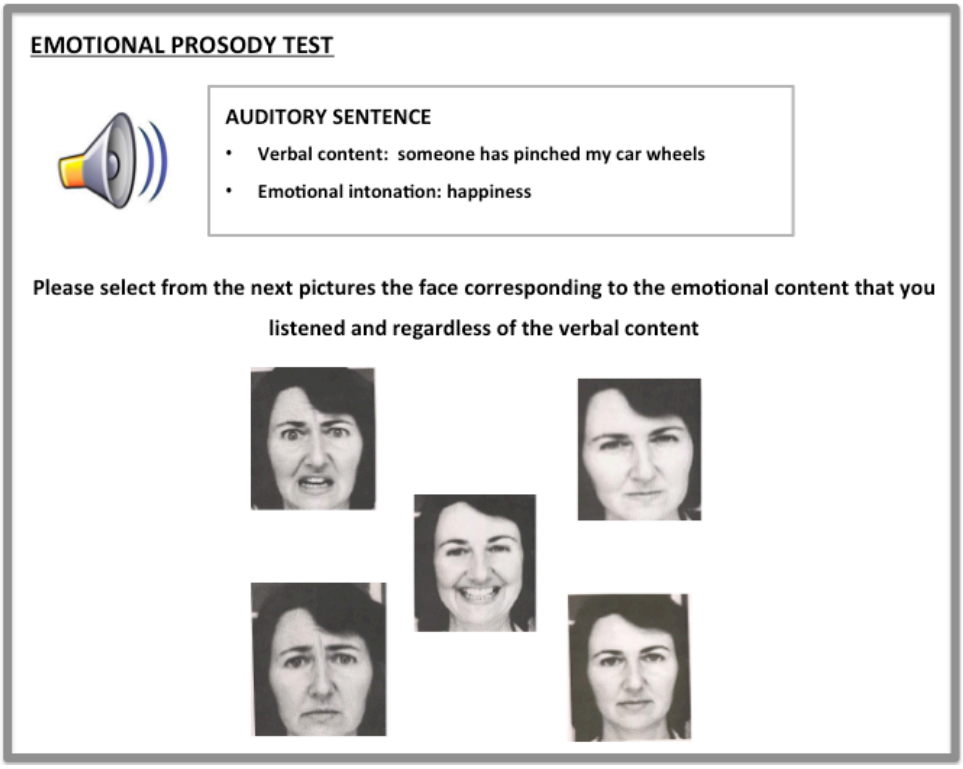
All the HIV+ participants and healthy adults individually performed the final prosody processing test. All listened nine different auditory sentences and were asked to select the emotional face corresponding to the emotional prosody, regardless of the verbal emotional content (Figure 9)

Table 8. Test creation phase and final selection of items. Percentage of agreement in nineteen prosody processing items (n=60 students)

Verbal/Prosody content (auditory items)	Prosody category response. N (%)						
	HAPPINESS	FEAR	SADNESS	NEUTRAL	SURPRISE	ANGER	OTHER/ NO ANWER
Neutral/happiness	44(73%)			5	11		
Sadness/ happiness	47(78%)		2	10			1
Anger/neutral				25(42%)		35	
Fear/fear*[4]	1	55(92%)	1	1	2		
Happiness /sadness*			56(93%)	2		1	1
Neutral/anger	5	2		13		38(63%)	2
Sadness/neutral*[1]				60(100%)			
Anger/anger	25	16	1	1	3	14(23%)	
Fear/happiness*	51(85%)	1		5		1	1
Happiness/happiness*[2]	54(90%)	2			4		
Neutral/neutral*[8]			1	53 (88%)		6	
Fear/neutral*		1	1	57 (95%)		1	
Neutral/sadness*[5]			60(100%)				
Happiness/neutral			7	44 (73%)		9	
Sadness/sadness*[3]			59 (98%)	1			
Happiness/anger*[9]	4	1		4		51(85%)	
Neutral/fear	1	45(75%)	1	6	4	2	1
Happiness/fear*[6]	2	52(86%)	1		3	1	1
Anger/happiness*[7]	60(100%)						

Table 8 shows number of students (15 men, 45 woman) who selected each type of emotional prosody. * Items with high agreement percentage: equal or above 85%. Grey shadows represent items selected for the final prosody test. Numbers after * correspond to the order of items in the final test.

Figure 9. Item 7 of the prosody recognition test



Brain imaging measures

A subset of 36 HIV+ individuals completed a 3-Tesla Magnetic Resonance scan in addition to the standard neuropsychological battery and the new design prosody processing test. Volumes of cerebral regions, levels of N-acetyl-aspartate (NAA) as a measure of neuronal integrity, and levels of Myo-inositol (MI) and Choline (CHO) as measures of brain inflammation were obtained by MRI and MRS. Images in 3D were adquired using a 3.0 Signa HDx scanner (GH Healthcare, Waukesha,WI), and were processed using the SPM8 software (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, University College of London, UK).

MRS biomarkers were calculated in voxels located in frontal white matter, frontal grey matter, basal ganglia and parietal lobes. Volumes of cerebral regions were measured in larger voxel as gray matter, white matter and cerebrospinal fluid as well as more localized voxels in: frontal

lobes, temporal lobes, parietal lobes, occipital lobes, cingulated, thalamus, caudate, putamen, pallidum, hippocampus and amygdale, separately on right and left areas.

Data analysis

Chi-square test for categorical variables and Student T-test for continuous were used to compare baseline characteristics between the whole HIV+ group (n=100) and the healthy adult group (n=46). Absolute and relative frequencies for categorical variables and median (IQR) for continuous variables were used to describe sample characteristics.

To explore the association between the new design test and MRI and MRS markers, we separately analysed cerebral volumes, brain inflammation markers (CHO, MI) and a neuronal integrity marker (NAA) in the subset of aviremic HIV+ patients (n=36) who completed the Cerebral Magnetic Resonance scan. We conducted multiple linear regressions with the prosody processing test score as independent variable and each cerebral biomarker as dependent variable in different brain areas. Sex, age and education level were tested as confounders and retained in the model if produced a change greater than 15% in coefficient for the association between the prosody test score and the cerebral biomarkers. In addition, NCI status was included in the final models to test whether the association between the our prosody processing test scores and cerebral measures persited regardless of the presence of NCI.

To determine whether the total HIV+ group (n=100) performed worse than the healthy adult group, differences in average test score were calculated by Student T-test. In addition, we compared HIV+ participants with and without NCI, those with active and non-active HCV, and patients receiving protease inhibitor in monotherapy or as part of triple therapy regimens.

All analyses were conducted using Stata (V.12.0, Stata Corporation, College Station, Texas, USA).

4.3.2. Results

Cerebral correlates of the emotional prosody test in aviremic HIV+ individuals

The subset of HIV+ individuals (n=36) that underwent the emotional prosody processing test and the Cerebral Magnetic Resonance scan included predominantly middle age Caucasian males. These patients had middle level of education and a good current immunological status. The prevalence of NCI (47.5%) was high owing to the inclusion criteria for the neuroimaging sub-study (Pérez-Valero, et al., 2014a)(Table 9). Sex was found to be a confounding variable for the association of different cerebral volumes and the prosody test score, thus for biological plausibility reasons (Schlaepfer et al., 1995) all models including cerebral volumes were adjusted by sex. No confounding variables were found for inflammatory markers.

Table 9. Baseline characteristics of the HIV+ participants: total sample (n=100) and subgroup that completed the cerebral magnetic resonance scan (n=36)

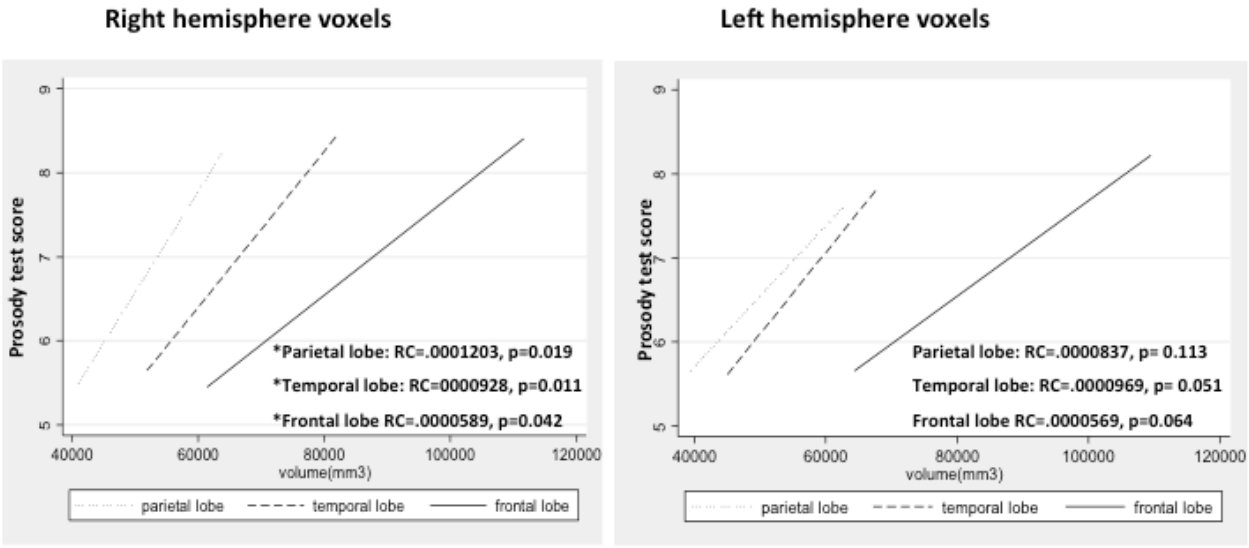
	Entire HIV+ sample (n=100)	HIV+ neuroimaging sub-group (n=36)
Caucasian. N (%)	88 (88)	31 (86.1)
Age. Median (IQR)	47.4 (43.1-51.9)	46.72 (40.7-49.4)
Years of education. Median (IQR)	10 (8-14)	9.0 (7.25-15)
AIDS diagnosis. N (%)	67 (67)	20 (55.6)
Mode of HIV transmission. N (%)		
Men who have sex with men	33 (33)	13 (36.1)
Heterosexual	29 (29)	12 (33.3)
Intravenous drug use	29 (29)	9 (25)
Vertical	9 (9)	2 (5.6)
Hepatitis C co-infection. N (%)		
No	56 (56)	25 (69.4)
Past	22 (22)	6 (16.7)
Active	22 (22)	5 (13.9)
Years since HIV diagnosis. Median (IQR)	16.7 (11.3-22.5)	14.2 (8.8-19.6)
Years on antiretroviral therapy. Median (IQR)	13.1 (7.4-16.8)	12.2 (6.2-15.1)
Years virologically suppressed. Median (IQR)	7.5 (4.4-10.2)	5.9 (3.6-9.6)
Current CD4 (cell/mm3). Median (IQR)	598 (406-720)	587 (468-712.8)
Nadir CD4 (cell/mm3). Median (IQR)	165.5 (50.3-268.5)	195 (75.3-291.5)
Neurocognitive impairment. N (%)	26 (26)	17 (47.5)

After adjusting for sex, we found a positive association between the emotional prosody test scores and several brain area volumes (Figure 10). Regarding cortical areas, right frontal, temporal and parietal lobes volumes had a statistically significant association with the test performance while the association with left hemisphere volumes was in the limit of significance. The magnitude of the effect showed a deterioration of between 0.6-1.2 points in prosody test for each 10,000-mm3 (10-cm3) reduction in the area volumes (Figure 10a). Regarding subcortical structures volumes, bilateral thalamus and left hippocampus had the strongest statistical association with the emotional prosody test scores (Figure 10b). Participants performed about one point worse in the prosody test per each 1,000 mm3 reduction in most of the subcortical areas significantly associated with the test performance. We did not find any association between the prosody test scores and overall grey, white matter or cerebrospinal volumes, occipital lobe, cingulate or other evaluated subcortical structures.

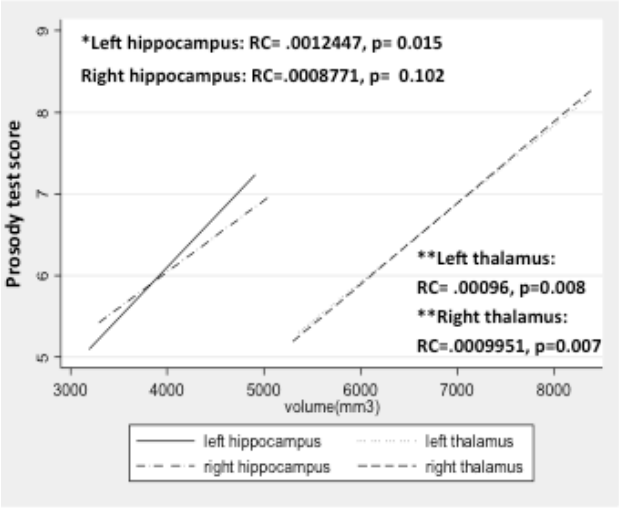
Regarding MRS markers, we found significant negative association between MI and CHO in frontal white matter and the test scores. Participants with higher levels of those inflammatory markers had lower prosody test performance. Increases of one milimol per liter of MI determined 1.4 point less in the prosody test while a quarter of milimol per liter of CHO increasing determined 2.37 point less (Figure 10c). Conversely levels of inflammatory markers in frontal grey matter, parietal lobe and basal ganglia did not show significant association with the prosody test scores.

Figure 10. Prosody test scores predicting Cerebral Magnetic Resonance measures on different brain regions in HIV+ infected participants (n=36)

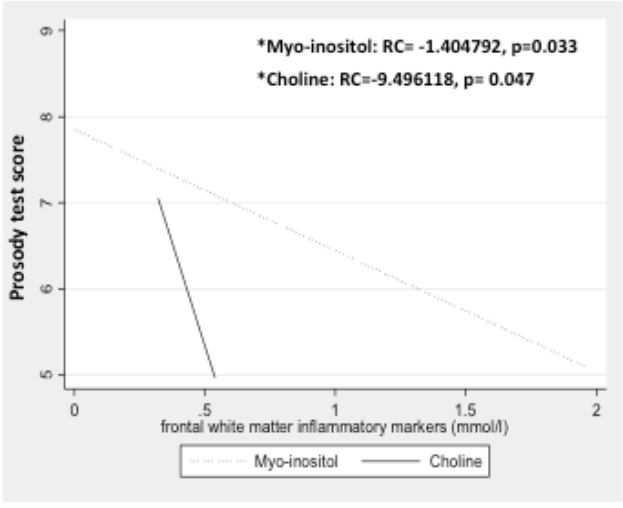
10a) Brain cortical volumes



10b) Brain subcortical volumes



10c) Frontal white matter brain inflammatory markers



Lines represent linear regressions between our prosody test scores and different cerebral measures. Regression coefficients (RC) show changes in the prosody test score for each unit of neuroimaging measure * p≤ 0.01; **p≤ 0.01. All regressions conducted with cerebral volumes as dependent variables were adjusted by sex.

We also were interested in exploring if the associations found between performance in the prosody test and cerebral measures persisted regardless of global NCI. To explore this association we forced to enter in each of the multiple linear regressions described above the patients' neurocognitive status. The effect of cerebral measures in the vocal emotion processing performance was reduced or abolished in some cases after adjusting by NCI. Regarding cortical areas, only right temporal (RC= .000073 (IC 95%, 7.70^{e-06}-0.0001383), p= .030) and parietal lobe volumes (RC= .0000918, (IC 95%, 1.73^{e-08}- 0.0001836), p=.050) persisted significantly associated to the prosody test scores. Statistical significance was reduced in right (RC= .0000453, (IC 95%, -6.31^{e-06}-0.0000969), p= .083) and left (RC=.000049 (IC 95%, -4.68^{e-06}-0.0001028), p= .072) frontal volumes after adjust by NCI. Bilateral thalamus and left hippocampus volumes also remained positively associated to the prosody test scores. After controlling by NCI, white matter inflammatory markers MI (RC= -.9540224, (IC 95%, -2.15219-0.2441456), p= 0.115) and CHO (RC= -3.960772, (IC 95%, -13.88422- 5.962672), p= 0.422) lost the association with the prosody test scores.

Vocal emotion processing deficits in aviremic HIV+ patients

We also tested differences in the prosody processing test performance between our sample of 100 aviremic HIV+ individuals and 46 healthy adults participants.

Middle age Caucasian males mostly composed the HIV+ sample. Most of them did not have active HCV infection although around a quarter had been treated successfully for HCV in the past. Twenty-six HIV+ patients (26%) had NCI (Table 9). The participants of the healthy adults group were also middle-aged (mean= 45.46 years), mostly men (65.2%) and did not differ from HIV+ participants in average age (*t* (146)= -1,158, *p*= .25) or gender proportion (χ^2 (1, N=146) = 0,918, p= .33).

To explore deficits in the processing of vocal emotional stimuli, we calculated differences in average raw scores by Student T-test between HIV+ participants and healthy adults. The HIV+ group (mean= 6.4; SD= 1.4) had overall lower performance than the healthy adults group (mean= 7; SD= 1.7) in the emotional prosody processing test ($t(144) = 2.07$, $p= .04$). A post hoc analysis revealed that HIV+ patients with NCI (mean= 5.7; SD= 1.4) performed significantly worse than adults of comparison ($t(71)= 3.19$, $p= .02$), although those patients without NCI (mean= 6.7; SD= 1.7) performed similar to the healthy adults group ($t(117) = 1.09$, $p=.28$).

Treatment type or HCV status had not effect over test scores. Patients with active HCV (mean= 6.2; SD=1.5) performed similar to patients who were successfully treated or who never had HCV (mean= 6.5; SD=1.4) ($t(98) = .91$, $p=.37$). Moreover, patients receiving protease inhibitor in monotherapy (mean= 6.4; SD=1.4) or as part of triple therapy regimens (mean= 6.3; SD=1.6) had not significant differences in the prosody test performance ($t(92) = -.46$, $p=.65$).

5. DISCUSSION

Our results provide new information on the neuropsychological functioning of HIV+ persons receiving effective ART in our health care context. To our knowledge our detailed neuropsychological study is the first to analyse functioning jointly in several cognitive, motor, and emotional processing measures in a group of aviremic HIV+ patients. In addition, having previously demonstrated that HIV+ patients on monotherapy did not have increased prevalence or incidence of NCI (Pérez-Valero et al., 2013; Pérez-Valero et al., 2014b), we now provide a more detailed estimation of the neuropsychological safety of using protease inhibitor monotherapy regimens.

Our results show that several neuropsychological deficits can persist in HIV+ patients, despite effective ART. Our data are consistent with the persistent cognitive, motor, and facial emotion recognition deficits previously described in HIV+ patients on ART. Moreover, we report the first data suggesting persistent deficits in the processing of vocal emotional stimuli. We believe that our results highlight the need for objective emotional processing tasks to be administered as a complement to traditional cognitive and motor tests. If further studies confirm our outcomes, testing emotional processing as part of neuropsychological assessments might improve our ability to detect neuropsychological disorders in HIV+ individuals.

Moreover, comparisons of neuropsychological patterns of functioning among HIV+ patients receiving protease inhibitors in monotherapy or as part of triple therapy provide reassurance on the neuropsychological safety of monotherapy regimens. In addition, our neuropsychological outcomes question the general clinical relevance of using ART regimens with different numbers of neuroactive drugs or different CPE score. However, using ART regimens with varying abilities to penetrate the CNS might be relevant for specific neuropsychological processes and in the subset of patients with NCI.

In the following section, we discuss the results of our three empirical analyses, their clinical relevance, and their implication for knowledge of the neuropsychological processes and neural substrates involved in the neuropsychological tasks performed. We also state the limitations of our analysis and discuss further research in the field.

The methods and results of the analyses conducted are summarized in Table 10.

Table 10. Overview of the empirical analyses conducted in the doctoral thesis

SHORT STUDY TITLE AND SAMPLE CHARACTERISTICS	PATIENTS, MATERIALS, AND METHODS	RESULTS
Analysis 1. Comparison between HIV+ patients on protease inhibitor (PI) monotherapy (MT) and on triple therapy (TT) including a PI. Cognitive/motor pattern in aviremic patients Total sample (n=191) ♦ HIV+ patients, aviremic (≥ 1year) ♦ Same ART regimen and virologically suppressed • MT group=96 • TT group=95 ♦ No major neurocognitive confounders	All patients underwent a neuropsychological (NP) assessment based on 14 measures covering 7 cognitive/motor domains Data analysis: Comparison between MT and TT groups in: • Average scores in all NP measures after adjusting for clinical and sociodemographic confounders • Frequency of deficits in all NP measures (mild and moderate deficits) Same group comparisons, only in patients with global neurocognitive impairment (NCI) Proportion of deficits in all HIV+ patients regardless of ART regimen	No differences in most NP measures between MT and TT groups Differences in average raw scores adjusted for confounders: ○ TT < MT: Verbal learning (p= .02, d= .28); Verbal recall (p= .02, d= .25) ○ MT < TT: fine motor skill dominant hand: (p=.01, d=.23) Differences in frequency of deficits. Only in moderate deficits: ○ TT > MT: Trail Making Test-A: (p= .03); Trail Making Test-B (p= .03) Similar differences, but with larger effect sizes (.047≥d≤.63) in patients with NCI Frequency of deficits in the whole HIV+ sample ○ Over 20%: digit symbol, visual learning, verbal delayed recall, fine motor skills, visual delayed recall, digit span. Frequency of deficits over 50% in the same measures for patients with NCI
Analysis 2. Facial emotion processing in aviremic HIV+ patients HIV+ sample (n=107) ♦ Receiving effective ART, aviremic for at least 2 years ♦ Excluding major neurocognitive confounders Healthy adults (HA) sample (n=40) ♦ Age and sex matched to HIV+ patients	All participants completed six tasks involving discrimination, memorization, and recognition of facial expressions Same NP assessment as in Analysis 1 (only in the HIV+ participants) Data analysis: Comparison between HIV+ and HA participants: • Average raw scores in all the emotional tasks performed • Distribution of correct response in recognition of specific emotions Medical/cognitive predictors of low accuracy in the recognition of specific emotions in the HIV+ group Comparison of the overall performance of each facial processing task between the following: HIV+ patients with NCI and without NCI; HCV–co-infected and non–HCV-co-infected; patients on MT and TT	HIV+ participants compared with the HA group: ○ Similar overall discrimination, memorization, and recognition ○ Worse recognition of sadness in the naming task (p<.05) ○ Worse recognition of sadness, anger, and fear in the selection task (p<.05) Predictors of low recognition accuracy in the HIV+ group: ○ Only WAIS-III Vocabulary scores predicted lower recognition of sadness and anger (p<.05) (only in the selection task). Medical and NCI did not predict lower scores HIV+ participants with and without NCI compared with HA: ○ Both memorization tasks: patients with NCI < non-NCI (p<.001, d>.80) ○ Facial affect naming recognition task: HIV+ participants with NCI < non-NCI (p=.06, d=.40)
Analysis 3. Impaired vocal emotion processing in aviremic HIV+ patients Student volunteers (n=90): test design phase HIV+ sample (n=100) ♦ Receiving effective ART, aviremic for at least 2 years Healthy adults sample (n=46) ♦ Age and sex matched to HIV+ patients	Test design phase: 30 student volunteers chose the emotional content from five written sentences; 60 student volunteers chose the emotional prosody content from 19 auditory items All the HIV+ and HA completed the final vocal emotion processing test Same NP assessment as in Analysis 1 (only in HIV+ participants) 36 HIV+ patients also underwent 3-T magnetic resonance imaging (MRI) Data analysis: Cerebral correlates of the prosody test scores in HIV+ participants (n=36), after adjusting for confounders. Separately, association after adjusting for NCI status HIV+ (n=100) and HA compared in average prosody test score HIV+ patients with NCI, HIV+ patients without NCI, and HA compared in prosody test performance	Nine items were selected for the final vocal emotion processing test Cerebral correlates of the prosody test (36 HIV+ participants) Poorer prosody test scores associated with poorer markers in: ○ Right frontal, temporal, and parietal lobes; bilateral thalamus and left hippocampus (p< .05) ○ Frontal white matter (higher inflammatory markers: choline and myo-inositol) (p< .05) Cerebral correlates of the prosody test regardless of NCI (100 HIV+ participants) ○ Lower right temporal lobe, right parietal lobe, bilateral thalamus, and left hippocampus volumes continued to be associated with poorer prosody test scores (p< .05) HIV+ participants compared with the HA group in the prosody test score: ○ Worse overall vocal emotion processing only confirmed in HIV+ patients with NCI (p< .001)

5.1. Neuropsychological pattern of functioning in aviremic HIV+ patients

5.1.1.Cognitive and motor functioning

The results of Analysis 1 showed that our selected HIV+ patients as a group had not large deviations from the expected neuropsychological values in the normative data for most measures. However, over 20% of patients had deficits in the following neuropsychological measures: digit symbol-coding and digit span (WAIS-III), visual learning and visual delayed recall (Brief Visuospatial Memory Test- Revised, BVMT-R), verbal delayed recall (VSRT), and fine motor skills (Grooved Pegboard Test) (Figure 5). More than 50% of patients with NCI had deficits in the same measures (Figure 6).

Since patients in the sample had few neurological comorbidities, good viral control, and high levels of adherence to ART, it seems plausible that the persistent neuropsychological deficits we found might be at least partially explained by the early irreversible or chronic effects of HIV on the brain. Moreover, because patients in our sample were receiving long-term treatment, the ability of treatment to penetrate the CNS might have contributed to the development or worsening of neuropsychological deficits.

To our knowledge, the cognitive and motor pattern of functioning has not been analysed in detail in highly adherent, aviremic HIV+ patients receiving long-term treatment. However, the deficits we found are compatible with neuropsychological outcomes previously described in HIV+ patients in the ART era. A small sample of HIV+ patients receiving triple therapy regimens had similar deficits to those we found in digit span, verbal learning, verbal delayed recall, symbol digit, and fine motor skills (Cysique, Maruff, & Brew, 2004a). Moreover, deficits in verbal learning, verbal delayed recall, and digit span have also been reported in an HIV+ sample of

aviremic but not all of those highly adherent patients (Ciccarelli et al., 2013). In contrast to our findings, none of those studies reported deficits in visual learning or delayed recall measures. However, data are not comparable because the authors did not report visual learning measures and estimated delayed recall performance using a different instrument, the Rey-Osterrieth Complex Figure test. Impairment in the learning and memory domains, including the Figure Memory Test, which is similar to the BVMT-R that we used, has previously been reported in a larger cohort of patients, although not all of those patients were receiving ART (Heaton et al., 2011). Despite similarities, samples are not directly comparable, because only 63% of patients in the study by Ciccarelli et al. (2013) had adherence above 80%, and the duration of virological suppression was not reported. Not all HIV+ patients in the study by Cysique et al. (2004) were aviremic despite being on ART.

Due to the multifaceted nature of the neuropsychological tasks, several processes and cerebral systems may be involved in deficits that we and other authors detected.

The WAIS-III digit symbol-coding task requires the patient to copy a symbol under a series of digits as fast as possible within a limited time. Speed of information processing, working memory, and motor functioning can interfere with test performance. Poorer levels of markers of the integrity of white matter and basal ganglia in an HIV+ sample on triple ART—not all aviremics—correlated with poorer test scores (Mohamed et al., 2010). Although improvements in a similar task were associated with viral suppression after starting ART (Sacktor et al., 2003), improvements in digit symbol were also reported in patients who discontinued their treatment (Robertson et al., 2010).

In the WAIS-III digit span subtest, the examiner read a series of numbers (eg. 1,2,5), and the participant had to repeat them immediately in the same order (digit forward) and in reverse order (digit backward). The test requires proper short-term memory process, attention, and

working memory abilities. Before the advent of ART, poorer forward digit span was associated with brain atrophy in HIV+ patients (Hestad et al., 1993).

We used the BVMT-T to assess visual memory. During three learning trials, patients had to learn the shape and position of six geometrical figures (10 second per learning trial). After 25 minutes, patients had to spontaneously remember the figures and their position. Poor performance in the BVMT-R may be due to visual learning or recall deficits. However, slow processing speed may be also involved in poor test outcomes because the learning time is limited in each trial (Tam & Schmitter-Edgecombe, 2013). In order to assess verbal memory, we used the VSRT, which follows similar procedures. Patients learn a list of 12 words in six learning trials, and after 20 minutes they have to spontaneously recall the words. Both verbal and visuospatial episodic memory performance have been associated with poorer integrity of white matter in the fornix and hippocampus in patients with Alzheimer disease, mild cognitive impairment (Sexton et al., 2010) and healthy volunteers (Rudebeck et al., 2009). A recent study found poorer scores in the BVMT-R and the Hopkins Verbal Learning Test-Revised (which is similar to the VSRT in terms of procedures) in HIV+ patients with poor levels of MRI/MRS markers in the hippocampus and parahippocampal gyrus (Wang, Wang, Ding, & Shang, 2015). Frontal lesions may also produce memory dysfunction (Kopelman & Stanhope, 1998) and executive function deficits, thus explaining in part the difficulties associated with memory tasks (Duff, Schoenberg, Scott, & Adams, 2005).

In the Grooved Pegboard Test, participants placed pegs into differently positioned slots. This manipulative dexterity test requires motor speed and complex visual motor coordination. Diffuse brain dysfunctions have been associated with the performance of this test (Theill, Martin, Schumacher, Bridenbaugh, & Kressig, 2011), and a diffuse pattern of white matter injury has been described in the HIV+ population (Gongvatana et al., 2011). In addition, grey matter

atrophy in the basal ganglia has been correlated with poorer test performance in HIV+ patients (Kuper et al., 2011). Improvements in the test scores have been observed after initiation of ART (Sacktor et al., 1999), and poor test performance was associated with an increased risk of incident dementia (Stern et al., 2001).

Given the multifaceted character of the tasks, it is difficult to draw conclusions on the predominant neuropsychological processes altered in the HIV+ sample studied here. Dysfunctions in the speed of processing, working memory, visual and verbal short-term memory, visual and verbal long-term memory, executive functions, motor speed, and visual motor coordination might be involved in the deficits observed. For this reason, it would be highly speculative to propose which neural substrates are associated with the resolution of each task. However, the deficits we found are consistent with those reported in other HIV+ cohorts and with selective impairment in fronto-striatal circuits (Heaton et al., 1995).

In summary, we believe that the cognitive and motor deficits observed in our sample might be at least partially explained by the past or current effect of HIV on the brain despite effective ART. Given the improvements in the performance of some tests after discontinuation of ART, we cannot rule out the possibility that neurotoxic effects contribute to increased deficits in specific neuropsychological measures.

5.1.2. Facial emotion processing

The results from Analysis 2 did not reveal worse performance in overall facial emotion recognition when we compared our sample of long-term aviremic HIV+ individuals with a group of healthy adults. There were no differences in facial discrimination or facial memorization tasks. Despite major similarities between the groups, the HIV+ group showed poorer recognition of sadness in the task that requires verbal labelling of emotion, and poorer recognition of sadness,

anger, and fear in the selection task that does not require verbal labelling.

Our results indicate overall preservation of facial emotion processing but selective subtle deficits in emotion recognition in aviremic HIV+ individuals. In our opinion, persistent subtle deficits might be associated with damage in emotion-related brain areas that is irreversible, even after long-term effective ART. Since all the patients in our sample had received long-term treatment, we cannot rule out neurotoxic effects of ART that could have contributed to the selective deficit in facial emotion recognition that we observed.

Previous studies reported no substantial abnormalities in overall discrimination and recognition of basic facial emotions in HIV+ patients, regardless of the effectiveness of ART. In agreement with Lane et al. (2012), but in contrast with Clark et al. (2010) and Baldonero et al. (2013), we found deficits in the recognition of sadness—but not of fear—in a naming test. Our findings of additional recognition deficits of sadness, anger, and fear are not comparable with the findings of previous studies, because, to our knowledge, ours is the first study in which a selection task that does not require verbal labelling of emotions has been performed. Because the selection task was easier than the naming task for both HIV+ participants and healthy adults, the deficits observed might indicate a higher sensitivity of the task to subtle abnormalities of recognition. Poorer reaction time for recognition of fear and poorer discrimination of the intensity of happy faces despite normal overall recognition in a naming task reported by Lane et al. (2012), might also suggest subtle alterations.

The differences between our results and those of previous studies might be related to the use and efficacy of ART. Both Clark et al. (2010) and Baldonero et al. (2013) reported fear recognition deficits in their HIV+ sample. Importantly, not all patients were receiving effective ART in either study. In contrast, impaired recognition of sadness and other subtle deficits have been reported in cohorts of HIV+ patients receiving ART, such as that of Lane et al. (2012) and ours. Fear

recognition deficits might be reversible upon initiation of ART or after a long period of effective ART, although sadness recognition deficits could remain. Mild persistent neurocognitive and motor dysfunction despite effective ART were also reported in our (González-Baeza et al., 2014; Pérez-Valero et al., 2013) and in others cohorts (Cysique & Brew, 2011a; Garvey et al., 2011).

The deficits in the recognition of negative emotions that we observed for the first time in an HIV+ sample are similar to those reported in patients with disconnection of the inferior fronto-occipital fasciculus, which is located in the temporal and frontal lobes along the anterior forceps and corpus callosum (Philippi, Mehta, Grabowski, Adolphs, & Rudrauf, 2009). Poorer recognition of sadness in Parkinson disease is also associated with impaired functioning of the inferior fronto-occipital fasciculus (Baggio et al., 2012). Activation of the amygdala is associated with processing of facial expression of fear and sadness (Adolphs, Tranel, Damasio, & Damasio, 1994); activation of the basal ganglia is associated with recognition of facial anger (Baggio et al., 2012; Calder, 2004). Our test data are consistent with compromised integrity of fronto-striatal and other axonal bundles, such as the posterior sectors of the corpus callosum (Pfefferbaum et al., 2009), and the reductions in subcortical volumes found in HIV+ patients. Specifically, the volumetric reduction of the amygdala, basal ganglia, and corpus callosum described in HIV+ individuals (Ances, Ortega, Vaida, Heaps, & Paul, 2012) might mediate the selective recognition deficits that we found in our HIV+ sample.

As secondary results, our analysis revealed that several variables were associated with processing of facial emotions. Although none of the markers of HIV progression or NCI status independently predicted recognition deficits for a specific emotion, active HCV reached an almost significant value as a predictor of poorer fear recognition in the selection task ($p = .052$). Otherwise, lower WAIS-III vocabulary subtest scores significantly predicted poorer recognition of sadness and anger in the selection task.

Fear recognition has been related to amygdala damage and white matter disconnection (Adolphs et al., 1994). HIV/HCV–co-infected individuals showed subcortical structure dysfunctions, especially in the basal ganglia but not in the amygdala (Garvey et al., 2012). An independent effect of HCV co-infection on white matter abnormalities has also been reported (Gongvatana et al., 2011; Jernigan et al., 2011). Therefore, more widespread disconnection of white matter fibres might mediate poorer fear recognition in the selection task in this subgroup of patients. Unfortunately, we cannot compare our data, because, to our knowledge, no previous studies have assessed processing of facial emotion in HCV-infected individuals.

The Vocabulary subtest has been used to estimate premorbid IQ and cognitive reserve. In the general population, low Vocabulary subtest scores have been associated with deficient neurocognitive performance (Corral, Rodríguez, Amenedo, Sánchez, & Díaz, 2006). In addition, aviremic HIV+ participants with lower estimated premorbid IQ more frequently had persistent neuropsychological deficits (Cysique & Brew, 2011a). Although confirmatory studies with more comprehensive measures of cognitive reserve are needed, we think that a low cognitive reserve might be associated with a higher risk of persistent deficits in recognition of facial emotions in HIV+ patients.

Moreover, although we did not find a significant independent effect of NCI status in any of the deficient processes, HIV+ patients with NCI had significantly worse overall facial memory performance than those without NCI. This subset of patients had a trend towards worse outcomes in the naming recognition task that requires verbal labelling of emotions ($p=0.06$) but not in the selection recognition task. In our opinion, patients with NCI might have more general recognition deficits than patients without NCI, particularly in situations requiring working memory. However, both deficits in emotion processing and cognitive/motor processes might have at least a partially different neural substrate, since NCI did not predict particular

recognition deficits in our HIV+ sample. The study by Lane et al. (2012), which included mostly virologically suppressed patients with a shorter time on ART than the patients in the present cohort, revealed significantly poorer overall recognition of facial emotion in a verbal labelling task in patients with NCI. The softer statistical effect found in our study might be associated with the benefit of longer virological suppression in processing recognition of facial emotion in patients with NCI.

In summary, our study results do not support a general trend toward poor discrimination, recognition, and memorization of facial emotion in aviremic HIV+ patients on effective ART. However, we do confirm the persistence of the sadness recognition deficits previously described in an HIV+ sample on ART. In addition, for the first time, we found recognition deficits in all the negative facial emotions assessed in a sample of HIV+ patients. Our results suggest that permanent damage of emotion-related brain systems might persist despite long-term effective ART, and that the neural substrate might be at least partially different from that of cognitive and motor dysfunctions. Permanent white matter dysfunction or reduction in the volume of subcortical structures such as the amygdala or basal ganglia, and corpus callosum could be involved in this type of deficit.

5.1.3.Emotional prosody processing

In Analysis 3, we used a new neuropsychological test to provide novel findings about vocal emotion processing in HIV+ people. The participants in the neuroimaging sub-study (n=36) with lower test scores had smaller volumes in all the lobes of the right hemisphere (except the occipital lobe), bilateral thalamus, and left hippocampus. They also had higher levels of inflammatory markers (CHO, MI) in frontal white matter. Smaller volumes in the right temporal and parietal lobes, bilateral thalamus, and left hippocampus remained significantly associated with lower test scores regardless of NCI. Furthermore, we found that the whole HIV+ sample

(n=100) had significantly worse test scores than healthy adults, but only in the subset of HIV+ patients with NCI.

Our results suggest that some HIV+ patients might have persistent deficits in emotional prosody processing despite long-term effective ART. Even though deficits in cognitive/motor and emotional prosody processing are found in patients with NCI, they could have a partially different structural basis in the brain. Compromise of the brain structures involved in the various steps of vocal emotion processing (Schirmer & Kotz, 2006) might explain the deficits observed in our aviremic HIV+ sample.

As for early auditory perception, we found lower thalamus volumes in patients with a poorer test performance. Of note, thalamic atrophy has been described in HIV+ individuals (Pfefferbaum et al., 2012). Thalamic regions are activated by both emotional and linguistic–non-emotional prosodic discrimination tasks (Wildgruber et al., 2004). Therefore, thalamic atrophy among HIV+ patients might lead to early impairment in auditory perception, regardless of emotional content. Further investigation is needed to determine whether HIV+ patients have generalized deficits in auditory discrimination that are not limited to emotional stimuli. Also in the early stages of auditory processing, the thalamus projects information to the temporal areas (Kolb & Whishaw, 2003) involved in the analysis of acoustic features and the synthesis of salient emotional information. Given our finding that lower right temporal volumes were associated with poorer test performance, we think that temporal lobe damage could also contribute to emotional prosody processing deficits in some HIV+ patients.

In later stages of processing, we found poorer test performance associated with lower frontal lobe volumes and higher levels of frontal white matter markers of inflammation in HIV+ patients. Brain alterations previously described in frontal regions of HIV+ individuals (Cysique et al., 2013; Harezlak et al., 2011) might also produce deficits shown by our test. Frontal dysfunction may be

involved in higher-order cognitive deficits, such as explicit judgment of affective voice or other cognitive demands of particular tasks (Schirmer & Kotz, 2006). Of note, the level of significance between bilateral frontal abnormalities and prosody test score was reduced after adjustment for NCI status.

The hippocampal atrophy previously described in HIV+ patients (Kallianpur et al., 2013) might also hinder the recognition of prosody in our test. The hippocampus appears to participate in the retrieval of complex high-resolution bindings (Yonelinas, 2013) and integration of auditory-visual stimuli (Joassin et al., 2010; Kirwan & Stark, 2004). The strong association we found between lower left hippocampus volumes and poorer test scores might be due to the demands of the task of matching emotional prosody to visual faces. Moreover, parietal lobe functioning has been associated with the auditory-visual integration process (Bernstein, Auer, Wagner, & Ponton, 2008) and with spatial auditory attention to emotional voice (Sander et al., 2005). Interestingly, we found a stronger association between right cortical volumes and test scores than between left cortical volumes and test scores. Predominant right hemispheric processing for extra-linguistic features (Kreitewolf, Friederici, & Kriegstein, 2014) and reduced emotional prosodic perception (Witteman, Van IJzendoorn, Van de Velde, Van Heuven, & Schiller, 2011) have been reported to be more frequent in right hemisphere damage than in left hemisphere damage.

Our results are not directly comparable with those of previous neuropsychological studies, because our study is the first to explore emotional prosody processing in an HIV+ sample. However, the ability to recognize emotions in the pictures of faces has been analysed elsewhere (Baldonero et al., 2013; Clark et al., 2010; 2015; Lane et al., 2012) and in our aviremic HIV+ sample (Analysis 2). Overall recognition of facial emotion was normal in our study and in the only published study in which all patients were receiving ART (Lane et al., 2012). However, in the study by Lane et al. (2012), patients with NCI had poorer overall recognition of facial emotion.

We found a similar trend in our study. Likewise, patients who were cognitively intact might have preserved recognition of emotional prosody, while patients with NCI might have prosody recognition deficits.

Taken together, our neuroimaging outcomes suggest that poorer test scores might result from damage in areas of the brain involved in different steps of the emotional processing of vocal stimuli. Because the association between our prosody test scores and various cerebral markers persisted regardless of the presence of NCI, the neural substrates of cognitive/motor and prosody processing deficits might be partially different. Moreover, it is plausible that the same early irreversible or chronic process of brain damage produced by HIV infection was responsible for both types of deficits. Of note, deficit in overall recognition appears to be limited to HIV+ patients with NCI in the present sample, which has few neurological comorbidities. Further studies should be conducted to clarify the steps in vocal emotion processing that are altered in HIV+ patients with prosody processing deficits.

5.2. Effect of using protease inhibitor monotherapy on neuropsychological functioning

The results of Analysis 1, which compared two groups of aviremic HIV+ patients in 14 cognitive and motor neuropsychological measures, revealed mainly similarities but also several differences between patients treated with a boosted protease inhibitor in monotherapy (n=95) or as part of triple therapy (n=96). Both groups tended to underperform in the same neuropsychological measures (ie, symbol digit, visual learning, visual delayed recall, verbal delayed recall, and motor skills). No differences were found between treatment groups in any of the facial emotion processing tasks of Analysis 2 or in the vocal emotion processing task of Analysis 3.

Despite general similarities, patients receiving triple therapy had significantly worse average verbal learning and verbal delayed recall scores, while those on monotherapy had worse average fine motor skills with the dominant hand. In addition, patients receiving triple therapy had slightly more frequent moderate deficits (≤ 2SD from the normative mean) in the Trail Making Test forms A and B. Similarly significant differences were found between the treatment groups, although larger effect sizes were found in the subset of patients with NCI (n=52). In addition, we found higher proportion of mild deficits in the measurement of motor skills with dominant hand in this subset of patients of with NCI in the monotherapy group.

In our opinion, most similarities contradict the notion that the pattern of neuropsychological functioning in patients receiving protease inhibitor in monotherapy differs from that of patients receiving protease inhibitors as part of standard triple therapy. The deficits we found in the whole HIV+ sample are compatible with the pattern of dysfunction previously described in HIV+ patients receiving triple ART (Ciccarelli et al., 2013; Cysique, Maruff, & Brew, 2004a; Heaton et al., 2011; Lane et al., 2012).

To our knowledge, no previous study has evaluated the safety of protease inhibitor monotherapy using detailed neuropsychological measures. However, data from two clinical trials did not reveal differences between monotherapy and triple therapy when global neurocognitive scores obtained from five neuropsychological measures were compared (Clarke et al., 2014; Winston et al., 2013). We also previously reported the absence of an association between monotherapy and higher frequencies of NCI in our cohort (Pérez-Valero et al., 2013). We subsequently observed that monotherapy regimens were not associated with a higher incidence of NCI after one year of follow-up (Pérez-Valero et al., 2014b).

Taken together, results for aviremic HIV+ patients suggest that monotherapy was not systematically associated with worse neuropsychological outcomes than standard triple therapy.

Irreversible brain damage at early stages of infection or the effect of chronic HIV infection on the brain might explain the similar pattern of dysfunction found in both groups. However, specific neuropsychological differences might be associated with selective differential brain effects of both types of regimens, particularly in patients with NCI.

In addition to the safety of monotherapy in the CNS, we discuss the consequences of using ART regimens with a higher or lower CPE score or number of neuroactive drugs. While there was concern about the effects of regimens with a low number of neuroactive drugs, including protease inhibitor monotherapy regimens, some studies found worse neurocognitive outcomes in ART regimens containing more neuroactive drugs and with a higher CPE score (Caniglia et al., 2014; Marra et al., 2009). The regimens that we compared had very different CPE scores. Protease inhibitor monotherapy comprised only one neuroactive drug—boosted darunavir or lopinavir—and had a CPE score of 3, while the triple therapy regimens had at least two neuroactive drugs and a CPE score of 7 (see Table 2).

In our opinion, most of the similarities found between treatment strategies and the small effect sizes of differences raise doubts about the clinical relevance of using one or more neuroactive drugs in well-treated, virologically suppressed patients. We believe that controlled peripheral viral load may be a good indicator of current neuroprotection, regardless of the number of neuroactive drugs contained in the ART regimen or its CPE score.

However, selective worse outcomes in verbal learning, verbal recall, and the Trail Making Test in the triple therapy group might be related to the effects of receiving ART regimens with a greater CPE score. Specific brain systems that sustain attentional, psychomotor speed (Trail Making Test forms A and B), cognitive flexibility (Trail Making Test form B), and verbal memory processes (VSRT) might be more vulnerable to the effects of excessive CNS penetration. Conversely, worse fine motor performance in the monotherapy group might be related to the negative effects of

using regimens with diminished ability to penetrate the CNS. Given the larger effect sizes, the clinical relevance of differences could be limited to the subgroup of patients with NCI.

To our knowledge, only two previous studies have compared individual neuropsychological results based on the number of neuroactive antiretroviral drugs or CPE score. No differences were found in most neuropsychological measures when patients on ART regimens with different numbers of neuroactive drugs were compared (Ciccarelli et al., 2013; Cysique, Maruff, & Brew, 2004a). However, both reported that patients with a higher number of neuroactive drugs performed better in few measures. Cysique et al. (2004) found differences in verbal memory measures—only in patients with NCI—while Ciccarelli et al. (2003) found differences in measures of visual delayed recall and sustained attention. Our results are not directly comparable with those reported elsewhere, because previous studies only included regimens with at least three drugs. Moreover, Cysique et al. (2004) also included patients who were not virologically suppressed and who were taking regimens with no neuroactive drugs. Based on our and previous results, we believe that the specific brain systems involved in memory, attention, cognitive flexibility, and fine motor tasks might be more vulnerable than other systems to the different degrees of CNS penetration by ART. The differences both we and Cysique et al. (2004) report suggest that the clinical impact of ART regimens with different abilities to penetrate the CNS might be relevant only in patients with NCI.

Consistent with results from other authors, we confirm that our sample of HIV+ patients receiving effective ART had similar persistent neuropsychological deficits regardless of the CPE score or the number of neuroactive drugs. Despite overall similarities, the cerebral and neuropsychological effects associated with the ability of the drug to penetrate the CNS might be relevant in patients with NCI.

5.3. Clinical implications of our empirical studies and future research

The results of our three empirical analyses make it possible to characterize the neuropsychological pattern of functioning in aviremic HIV+ patients receiving stable ART. We confirmed the persistence of previously reported cognitive, motor, and emotional processing dysfunctions and we propose new, potentially persistent deficits. In our opinion, the deficits we found in a sample with few neurological comorbidities support the notion that the early persistent or chronic effect of HIV infection might affect brain systems that sustain cognitive, motor, and emotional processes despite effective ART. Moreover, because patients in our sample were receiving long-term treatment, some deficits may have been associated with the ability of treatment to penetrate the CNS. The alteration in all the neuropsychological measures we found may be associated with the fronto-striatal and subcortical structure dysfunctions that are characteristic of HIV+ patients. However, since our tasks could involve multiple neuropsychological processes, the involvement of other brain systems cannot be ruled out.

In our opinion, these results highlight the need for broad characterization of the persistent neuropsychological deficits that we can expect in well-treated HIV+ individuals. In particular, the independence between cognitive/motor and emotional processing deficits that we found emphasizes the importance of studying emotional processing in HIV+ individuals.

New confirmatory longitudinal studies with larger and more representative sample sizes, and better-characterized groups of healthy adults are necessary to confirm our findings. More extensive emotional assessments, including reaction time, recognition of additional emotions, and auditory discrimination measures, could prove useful for detecting deficits as yet unknown among HIV+ individuals. Additional cognitive tasks, such as planning tasks in the executive functioning domain (eg, Tower of London) or selective/sustained attention (eg, the d2 test),

would lead to more solid conclusions about the neuropsychological processes altered in this population. In addition, more complex tasks that cover day-to-day demands (eg, facial recognition from 3D stimuli) could generate more data on the impact of deficits in real life. Furthermore, new and more comprehensive neuroimaging techniques, such as diffusion tensor imaging or functional MRI, would facilitate identification of the structural damage associated with deficits.

In our opinion, if future studies confirm the emotional processing deficits in HIV+ patients, the next revision of the current criteria for HAND diagnosis (Antinori et al., 2007) should consider the inclusion of the emotional processing as an additional domain to be assess for global HIV-neuropsychological disorder detection.

Some of the data reported in Analysis 1 reinforce previous evidence in our and other cohorts, which supports the neurologic safety of using protease inhibitor monotherapy in aviremic HIV+ patients. Our study questions the clinical relevance of using ART regimens with differing numbers of neuroactive drugs. The similar pattern observed regardless of the type of treatment leads us to hypothesize that ART regimens that achieve peripheral viral suppression provide similar overall protection of the brain and neuropsychological function. Furthermore, the selective differences we found lead us to believe that ART regimens might exercise specific brain and neuropsychological effects depending on their ability to penetrate brain tissue. These differential effects would be limited to patients with NCI. If our hypotheses are confirmed, the suppression of plasma viral load might be used as an estimator of overall neuropsychological protection. Moreover, using ART regimens with optimal CNS penetration might go some way to maximizing neuropsychological functioning in the subset of patients with NCI.

Further clinical trials should analyse the CNS penetration and neurotoxicity profiles of specific ART regimens. Comparisons of detailed neuropsychological measures and markers in

neuroimaging or cerebrospinal fluid might enable us to construct ART regimens that achieve an optimal balance between CNS penetration and neurotoxicity. Such studies could minimize neuropsychological deficits among HIV+ patients and be particularly relevant in HIV+ patients with NCI.

5.4. Strengths and limitations of our empirical analyses

Our results are strengthened by the comprehensive neuropsychological assessment we performed based on expert recommendations to detect NCI in HIV+ patients. The neuropsychologist who conducted the assessment was blind to the patients' clinical features and treatment. A physician selected all candidates for inclusion in the study and recorded the medical variables from the clinical history. We also followed an exhaustive preliminary selection process to exclude patients with major neurocognitive confounders, including clinical interviews to detect major depressive disorders. All patients had been virologically suppressed for at least one year at inclusion and for at least two years at the follow-up visit. Furthermore, we adjusted the test results for sociodemographic and clinically relevant variables to improve the value of our conclusions. In addition to the behavioural outcomes we reported, Analysis 3 included several brain correlates obtained by MRI.

Our three analyses are subject to limitations that are inherent to all cross-sectional studies. One such limitation is selection bias. Patients who agreed to participate in our study might have had lower frequencies of NCI than patients who refused to participate; consequently, a higher proportion of deficits might be expected in well-treated HIV+ patients. However, we believe that this is unlikely because of our study criteria, which stipulated only long-term virologically suppressed patients with few comorbid CNS conditions. This type of patient had a low risk of NCI.

Other major limitations of our study are those arising from the neuropsychological assessment conducted in all the empirical studies of the doctoral thesis. Although we used demographically corrected norms, statistical adjustment to control for confounding variables, and healthy adult comparison groups when available, the unavailability of extensive normative studies of neuropsychological tasks in people living in Spain could worsen our estimations of deficits. Normative studies or larger sample sizes in the healthy adult comparison groups, including magnetic resonance imaging data, would allow for better control of confounding variables.

The multifaceted character of the neuropsychological tasks used does not allow us to draw precise conclusions about abnormalities in neuropsychological processes or neural substrates. Comprehensive neuroimaging measures, including those obtained with functional MRI, would have helped to determine the brain networks implicated in the deficits found. Furthermore, the neuropsychological measures we used are limited, and additional measures could have been more sensitive to the effect of variables such as ART-induced neurotoxicity. Moreover, we did not include measures to assess the daily impact of neuropsychological deficits, which would have helped to determine the clinical significance of our data.

Our results are limited to our sample of aviremic patients who were receiving protease inhibitors in monotherapy and administered as part of triple therapy. In addition, the study patients had a good immunological status and had been virologically suppressed for several years. Future studies should be performed in a more representative sample of treated patients, given that many other drug combinations are prescribed in the HIV health care context. We also included participants with active HCV infection, because we were interested in studying a representative sample of patients in Spain, although we are aware this might confound conclusions about the role of HIV infection in patterns of neuropsychological functioning.

6. CONCLUSSIONS/CONCLUSIONES

6.1. Conclusions: English version

1. Cognitive and motor deficits appear to persist in selected HIV+ patients receiving effective ART in our health care context. The highest frequencies of impairment were observed for tasks that require verbal and visual memory, attention/working memory, executive function, fine motor skills, and speed of information processing.
2. Our data suggest that patients studied here can distinguish between different facial emotions and preserve overall recognition of facial expression, in much the same way as patients from clinically heterogeneous cohorts. These processes appear to be preserved regardless of ART and viral control in HIV+ patients.
3. Patients in this study present selective recognition deficits for basic negative emotions. These recognition deficits occur regardless of NCI and could be associated with white matter fibre disconnection and reduced volume of subcortical structures previously described in HIV+ patients.
4. Patients with NCI did not have overall facial emotion recognition deficits, although they did tend to show worse performance in one the recognition tasks that requires verbal labelling of emotions. Significant poorer recognition has been reported in patients with NCI but with a shorter time on ART. The softer statistical effect that we found might be associated with the benefit of longer virological suppression in patients with NCI.
5. Our data suggest that HIV+ patients without NCI have preserved vocal emotional processing. In contrast, patients with NCI appear to have deficits in this type of processing.
6. Although emotional prosody processing and cognitive/motor deficits seem to co-occur in our sample, brain volumes in several cortical and subcortical regions correlate with prosody test

performance, regardless of the presence of NCI. Both vocal emotion processing and cognitive and motor processes might be independently impaired.

7. The study patients had similar patterns of neuropsychological functioning regardless of whether they were receiving protease inhibitors in monotherapy or as part of triple therapy. Monotherapy regimens do not appear to be systematically associated with worse neuropsychological outcomes. Differences found in specific neuropsychological tasks by treatment group suggest that each type of regimen might have different effects on specific brain systems, although only in patients with NCI.
8. Similar neuropsychological patterns in an aviremic HIV+ sample receiving various ART strategies suggest that control of peripheral viral load may be a good indicator of neuroprotection, regardless of the number of neuroactive drugs or the CPE score. The few differences found by treatment group—limited to patients with NCI—suggest that excessive or deficient penetration of the CNS by ART might contribute to the development of selective neuropsychological deficits.
9. Our data suggest that some aviremic HIV+ patients might have specific deficits in cognitive and motor processes, facial emotion processing, and emotional prosody processing. It is plausible that HIV-induced brain damage persists despite the prolonged efficacy of ART. However, neuropsychological deficits may be due in part to scant or excessive penetration of the CNS by ART in patients with NCI.

6.2. Conclusiones: versión en español

1. Algunos déficits cognitivos y motores parecen persistir en nuestra muestra de pacientes infectados por VIH. Las tareas que requieren memoria verbal y visual, atención/memoria de trabajo, funciones ejecutivas, habilidades motoras finas y velocidad de procesamiento de la información presentaron mayor frecuencia de déficits.

2. Nuestros datos sugieren que los pacientes de nuestra muestra son capaces de discriminar distintas expresiones faciales y de modo global preservan el reconocimiento de expresiones faciales. Esos resultados son similares a los encontrados en otras muestras de pacientes clínicamente diferentes. Ambos procesos parecen estar conservados independientemente de si los pacientes reciben TAR y del control del virus.
3. Nuestra muestra de pacientes VIH parece presentar déficits específicos en el reconocimiento de las emociones básicas negativas. Dichos déficits parecen presentarse con independencia del estado cognitivo de los pacientes. La desconexión de fibras de sustancia blanca y las reducciones volumétricas de estructuras subcorticales previamente descritas en población VIH podría tener relación con los déficits descritos.
4. Los pacientes con cuadros de deterioro neurocognitivo no presentaron déficits generalizados reconociendo emociones faciales, pero tendieron a mostrar peores resultados que los pacientes no deteriorados en la tarea de reconocimiento que requiere etiquetado verbal de las emociones. Resultados similares con mayor significación estadística han sido descritos en otra cohorte de pacientes en menor tiempo en TAR. El menor efecto estadístico en nuestra muestra, podría estar relacionado con el beneficio de un mayor tiempo de supresión viral en los pacientes con deterioro neurocognitivo.
5. Nuestros datos sugieren que el procesamiento de emociones en voces está preservado en los pacientes VIH sin deterioro neurocognitivo. Por el contrario, los pacientes con cuadros de deterioro neurocognitivo parecen tener déficits generalizados en el procesamiento de prosodia emocional.
6. A pesar de que los déficits de procesamiento de prosodia y los cognitivos/motores parecen concurrir en nuestra muestra, menores volúmenes cerebrales de varias regiones corticales y subcorticales se asociaron con peores resultados en el test de prosodia después de ajustar

por el estado cognitivo. El procesamiento de prosodia emocional podría estar alterado con independencia de los procesos cognitivos y motores.

7. Los pacientes VIH avirémicos de nuestra muestra presentaron un patrón similar de funcionamiento neuropsicológico, independientemente de si recibían inhibidores de la proteasa en monoterapia o en terapia triple. Los regímenes de monoterapia no parecieron asociarse de manera sistemática a peores resultados neuropsicológicos. Las diferencias específicas encontradas entre grupos sugieren que cada tipo de régimen podría tener diferentes efectos sobre determinados sistemas cerebrales y, únicamente en pacientes con deterioro neurocognitivo.
8. El perfil similar mostrado por todos pacientes de nuestra muestra, sugiere que el control de la carga viral periférica podría ser un buen indicador de protección cerebral con independencia del número de fármacos neuroactivos o el "CPE score" de cada régimen. Las diferencias específicas entre grupos de tratamiento- limitadas a los pacientes con deterioro neurocognitivo- sugieren que, una escasa o excesiva neuropenetración podría contribuir al desarrollo de déficits neuropsicológicos específicos en los pacientes con deterioro cognitivo.
9. Nuestros datos sugieren que algunos pacientes avirémicos podrían presentar déficits específicos de procesamiento de emoción facial, de procesamiento de prosodia emocional, cognitivos y motores. Es posible que el daño cerebral inducido por VIH persista a pesar de la eficacia prologada de TAR. Sin embargo, tanto una escasa como una excesiva penetración en SNC podría estar explicando parte de los déficits neuropsicológicos encontrados, principalmente en los pacientes con cuadros de deterioro neurocognitivo

7. REFERENCES

Adolphs, R. (2002). Neural systems for recognizing emotion. Current Opinion in Neurobiology, 12(2), 169–177. doi:10.1016/S0959-4388(02)00301-X

Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. (1994). Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature*, *372*(6507), 669–672. doi:10.1038/372669a0

Allavena, C., Guimard, T., Billaud, E., la Tullaye, de, S., Reliquet, V., Pineau, S., … Raffi, F. (2014). Prevalence and risk factors of sleep disturbances in a large HIV-infected adult population. *Journal of the International AIDS Society*, *17*(4(Suppl 3)), 19576. doi:10.7448/IAS.17.4.19576

Ances, B. M., Ortega, M., Vaida, F., Heaps, J., & Paul, R. (2012). Independent effects of HIV, aging, and HAART on brain volumetric measures. *Journal of Acquired Immune Deficiency Syndromes*, *59*(5), 469–477. doi:10.1097/QAI.0b013e318249db17

Antinori, A., Arendt, G., Becker, J. T., Brew, B. J., Byrd, D. A., Cherner, M., ...Wojna, V.E. (2007). Updated research nosology for HIV-associated neurocognitive disorders. *Neurology*, *69*(18), 1789–1799. doi:10.1212/01.WNL.0000287431.88658.8b

Arribas, J. R., Horban, A., Gerstoft, J., Fätkenheuer, G., Nelson, M., Clumeck, N., …Moecklinghoff, C. (2010). The MONET trial: darunavir/ritonavir with or without nucleoside analogues, for patients with HIV RNA below 50 copies/ml. *AIDS*, *24*(2), 223–230. doi:10.1097/QAD.0b013e3283348944

Bagby, R. M., & Taylor, G. J. (1997). Affect dysregulation and alexithymia. In G. J. Taylor, R. M. Bagby, & J. D. Parker, *Disorders of affect regulation. Alexithymia in medical and psychiatric illness* (pp. 26–45). Cambridge: Cambridge University Press.

Baggio, H. C., Segura, B., Ibarretxe-Bilbao, N., Valldeoriola, F., Marti, M. J., Compta, Y., ...Junqué C. (2012). Structural correlates of facial emotion recognition deficits in Parkinson's disease patients. *Neuropsychologia*, *50*(8), 2121–2128. doi:10.1016/j.neuropsychologia.2012.05.020

Baldonero, E., Ciccarelli, N., Fabbiani, M., Colafigli, M., Improta, E., D'Avino, A., ...Silveri, M.C. (2013). Evaluation of emotion processing in HIV-infected patients and correlation with cognitive performance. *BMC Psychology*, *1*(1), 3. doi:10.1186/2050-7283-1-3

Bayon, C., Hernando, M. A., Álvarez, M. J., Pulido, F., Montesinos, F., Gutiérrez, F., ...Pulido, F. (2014). Comparación de dos medidas de screening para detectar depresión en pacientes con infección VIH (P-055). *Enfermedades Infecciosas Y Microbiología Clínica*, *32 (Especial Congreso 2)*, 52–53.

Benedict, R. H. (1997). *Brief Visuospatial Memory Test-Revised*. Odessa, FL: Psychological Assessment Resources,Inc.

Bernstein, L. E., Auer, E. T., Wagner, M., & Ponton, C. W. (2008). Spatiotemporal dynamics of audiovisual speech processing. *NeuroImage*, *39*(1), 423–435. doi:10.1016/j.neuroimage.2007.08.035

Bogdanova, Y., Díaz-Santos, M., & Cronin-Golomb, A. (2010). Neurocognitive correlates of alexithymia in asymptomatic individuals with HIV. *Neuropsychologia*, *48*(5), 1295–1304. doi:10.1016/j.neuropsychologia.2009.12.033

Bowers, D., Blonder, L., & Heilman, K. (1991). *The Florida Affect Battery*. Gainesville: Univ. of Florida.

Bucks, R. S., & Radford, S. A. (2004). Emotion processing in Alzheimer's disease. *Aging & Mental Health*, *8*(3), 222–232. doi:10.1080/13607860410001669750

Bunupuradah, T., Chetchotisakd, P., Jirajariyavej, S., Valcour, V., Bowonwattanuwong, C., Munsakul, W., ...HIV STAR Study Group. (2012). Neurocognitive impairment in patients randomized to second-line lopinavir/ritonavir-based antiretroviral therapy vs. lopinavir/ritonavir monotherapy. *Journal of NeuroVirology*, *18*(6), 479–487. doi:10.1007/s13365-012-0127-9

Buxton, S. L., MacDonald, L., & Tippett, L. J. (2013). Impaired recognition of prosody and subtle emotional facial expressions in Parkinson's disease. *Behavioral Neuroscience*, *127*(2), 193–203. doi:10.1037/a0032013

Calder, A. J. (2004). Impaired recognition of anger following damage to the ventral striatum. *Brain*, *127*(9), 1958–1969. doi:10.1093/brain/awh214

Caniglia, E. C., Cain, L. E., Justice, A., Tate, J., Logan, R., Sabin, C., …HIV-CAUSAL Collaboration. (2014). Antiretroviral penetration into the CNS and incidence of AIDS-defining neurologic conditions. *Neurology*, *83*(2), 134–141. doi:10.1212/WNL.0000000000000564

Carvajal, F., Rubio, S., Martín, P., Serrano, J. M., & García-Sola, R. (2009). Perception and recall of faces and facial expressions following temporal lobectomy. *Epilepsy & Behavior*, *14*(1), 60–65. doi:10.1016/j.yebeh.2008.08.016

Castellon, S. A., Hinkin, C. H., & Myers, H. F. (2000). Neuropsychiatric disturbance is associated with executive dysfunction in HIV-1 infection. *Journal of the International Neuropsychological Society*, *6*(3), 336–347.

Castellon, S. A., Hinkin, C. H., Wood, S., & Yarema, K. T. (1998). Apathy, depression, and cognitive performance in HIV-1 infection. *Journal of Neuropsychiatry*, *10*(3), 320–329.

Cherner, M., Ellis, R. J., Lazzaretto, D., Young, C., Mindt, M. R., Atkinson, J. H., ...HIV Neurobehavioral Research Center Group. (2004). Effects of HIV-1 infection and aging on neurobehavioral functioning: preliminary findings. *AIDS*, *18 (1)*, S27–34.

Ciccarelli, N., Fabbiani, M., Colafigli, M., Trecarichi, E. M., Silveri, M. C., Cauda, R., ...Di Giambenedetto, S. (2013). Revised central nervous system neuropenetration-effectiveness score is associated with cognitive disorders in HIV-infected patients with controlled plasma viraemia. *Antiviral Therapy*, *18*(2), 153–160.

doi:10.3851/IMP2560

Ciccarelli, N., Fabbiani, M., Di Giambenedetto, S., Fanti, I., Baldonero, E., Bracciale, L., …Silveri, M.C. (2011). Efavirenz associated with cognitive disorders in otherwise asymptomatic HIV-infected patients. *Neurology*, *76*(16), 1403–1409. doi:10.1212/WNL.0b013e31821670fb

Clark, U. S., Cohen, R. A., Westbrook, M. L., Devlin, K. N., & Tashima, K. T. (2010). Facial emotion recognition impairments in individuals with HIV. *Journal of the International Neuropsychological Society*, *16*(6), 1127–1137. doi:10.1017/S1355617710001037

Clark, U. S., Walker, K. A., Cohen, R. A., Devlin, K. N., Folkers, A. M., Pina, M. J., & Tashima, K. T. (2015). Facial emotion recognition impairments are associated with brain volume abnormalities in individuals with HIV. *Neuropsychologia*, *70*, 263–271. doi:10.1016/j.neuropsychologia.2015.03.003

Clarke, A., Johanssen, V., Gerstoft, J., Clotet, B., Ripamonti, D., Murungi, A., …Moecklinghoff, C. (2014). Analysis of neurocognitive function and CNS endpoints in the PROTEA trial: darunavir/ritonavir with or without nucleoside analogues. *Journal of the International AIDS Society*, *17*(4 (Suppl 3)), 19526. doi:10.7448/IAS.17.4.19526

Clifford, D. B. (2008). HIV-associated neurocognitive disease continues in the antiretroviral era. *Topics in HIV Medicine, 16*(2), 94–98.

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences* (2nd ed.). Hillsdale, NJ: Laurence Erlbaum Associates.

Cole, M. A., Castellon, S. A., Perkins, A. C., Ureno, O. S., Robinet, M. B., Reinhard, M. J., ...Hinkin, C.H. (2007). Relationship between psychiatric status and frontal–subcortical systems in HIV-infected individuals. *Journal of the International Neuropsychological Society*, *13*(03), 549-54. doi:10.1017/S135561770707066X

Corral, M., Rodríguez, M., Amenedo, E., Sánchez, J. L., & Díaz, F. (2006). Cognitive reserve, age, and neuropsychological performance in healthy participants. *Developmental Neuropsychology*, *29*(3), 479–491. doi:10.1207/s15326942dn2903_6

Cysique, L. A., Maruff, P., & Brew, B. J. (2004a). Antiretroviral therapy in HIV infection: are neurologically active drugs important?. *Archives of Neurology*, *61*(11), 1699–1704. doi:10.1001/archneur.61.11.1699

Cysique, L. A., & Brew, B. J. (2011a). Prevalence of non-confounded HIV-associated neurocognitive impairment in the context of plasma HIV RNA suppression. *Journal of NeuroVirology*, *17*(2), 176–183. doi:10.1007/s13365-011-0021-x

Cysique, L. A., Maruff, P., & Brew, B. J. (2004b). Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus–infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre- and post-highly active antiretroviral therapy eras: A combined study of two cohorts Clinical

Report. *Journal of NeuroVirology*, *10*(6), 350–357. doi:10.1080/13550280490521078

Cysique, L. A., Moffat, K., Moore, D. M., Lane, T. A., Davies, N. W. S., Carr, A., ...Rae, C. (2013). HIV, Vascular and Aging Injuries in the Brain of Clinically Stable HIV-Infected Adults: A 1(H) MRS Study. *PLoS ONE*, *8*(4), e61738. doi:10.1371/journal.pone.0061738

Cysique, L. A., Waters, E. K., & Brew, B. J. (2011b). Central nervous system antiretroviral efficacy in HIV infection: a qualitative and quantitative review and implications for future research. *BMC Neurology*, *11*(1), 148. doi:10.1186/1471-2377-11-148

Díaz, A., Sobrino, P., del Amo, J., Moreno, S., Díez, M., & Grupos de Trabajo de SINIVIH Epi-ITS Encuesta Hospitalaria y CoRIS. (2014). La cascada de tratamiento en España: primeras estimaciones (P-081). *Enfermedades Infecciosas Y Microbiología Clínica*, *32 (Especial Congreso 2)*, 63.

Díez, M., Oliva, J., Sánchez, F., Vives, N., Cevallos, C., Izquierdo, A., & Grupo SINIVIH. (2012). Incidence of new HIV diagnoses in Spain, 2004-2009. *Gaceta Sanitaria*, *26*(2), 107–115. doi:10.1016/j.gaceta.2011.07.023

Duff, K., Schoenberg, M. R., Scott, J. G., & Adams, R. L. (2005). The relationship between executive functioning and verbal and visual learning and memory. *Archives of Clinical Neuropsychology*, *20*(1), 111–122. doi:10.1016/j.acn.2004.03.003

Ellis, R. J., Badiee, J., Vaida, F., Letendre, S., Heaton, R. K., Clifford, D., ...CHARTER Group. (2011). CD4 nadir is a predictor of HIV neurocognitive impairment in the era of combination antiretroviral therapy. *AIDS*, *25*(14), 1747–1751. doi:10.1097/QAD.0b013e32834a40cd

Ellis, R. J., Gamst, A. C., Capparelli, E., Spector, S. A., & Hsia, K. (2000). Cerebrospinal fluid HIV RNA originates from both local CNS and systemic sources. *Neuro*logy, 54 (4), 927-36. doi:10.1212/WNL.54.4.927

Expert Panel of European AIDS Clinical Society. (2014). European AIDS Clinical Society Guidelines. Retrieved August 24, 2015, from http://scholar.google.com/scholar?q=related:UP7DDGiUMu0J:scholar.google.com/&hl=en&num=20&as_sdt=0,5&as_ylo=2014&as_yhi=2014

Expert Panel of GESIDA and the National AIDS Plan. (2015). Executive summary of the GESIDA/National AIDS Plan Consensus Document on antiretroviral therapy in adults infected by the human immunodeficiency virus (updated January 2015). *Enfermedades Infecciosas Y Microbiología Clínica*, *32*(7), 447–458. doi:10.1016/j.eimc.2015.03.017

Ferretti, F., Gisslén, M., Cinque, P., & Price, R. W. (2015). Cerebrospinal Fluid HIV Escape from Antiretroviral Therapy. *Current HIV/AIDS Reports*, *12*(2), 280–288. doi:10.1007/s11904-015-0267-7

Fields, R. D. (2009). *The other brain*: *From dementia to schizophrenia, how new discoveries about the brain are revolutionizing medicine and science.* New York: Simon & Schuster.

Garvey, L. J., Pavese, N., Ramlackhansingh, A., Thomson, E., Allsop, J. M., Politis, M., ...Winston, A. (2012). Acute HCV/HIV coinfection is associated with cognitive dysfunction and cerebral metabolite disturbance, but not increased microglial cell activation. *PLoS ONE*, *7*(7), e38980. doi:10.1371/journal.pone.0038980

Garvey, L., Surendrakumar, V., & Winston, A. (2011). Low rates of neurocognitive Impairment are observed in neuro-Asymptomatic HIV-Infected subjects on effective antiretroviral therapy. *HIV Clinical Trials*, *12*(6), 333–338. doi:10.1310/hct1206-333

Golden, C. J., & Freshwater, S. M. (2002). *Stroop color and word test: A manual for clinical and experimental uses*. Wood Dale, IL: Stoetling Company.

Gongvatana, A., Cohen, R. A., Correia, S., Devlin, K. N., Miles, J., Kang, H., ...Tashima, K.T. (2011). Clinical contributors to cerebral white matter integrity in HIV-infected individuals. *Journal of NeuroVirology*, *17*(5), 477–486. doi:10.1007/s13365-011-0055-0

González-Baeza, A., Carvajal, F., Bayón, C., Pérez-Valero, I., Estébanez, M., Bernardino, J. I., ...Arribas, J.R. (2014). Pattern of neurocognitive function in patients receiving boosted protease inhibitor monotherapy: a detailed neuropsychological study. *Journal of NeuroVirology*, *20*(4), 362–370. doi:10.1007/s13365-014-0251-9

González-García, J. J., Mahillo, B., Hernández, S., Pacheco, R., Diz, S., García, P., …Vázquez-Rodríguez, J.J. (2005). Prevalences of hepatitis virus coinfection and indications for chronic hepatitis C virus treatment and liver transplantation in Spanish HIV-infected patients. The GESIDA 29/02 and FIPSE 12185/01 Multicenter Study. *Enfermedades Infecciosas Y Microbiología Clínica*, *23*(6), 340–348.

Gutiérrez, F., García, L., Padilla, S., Álvarez, D., Moreno, S., Navarro, G., …CoRIS. (2014). Risk of clinically significant depression in HIV-infected patients: effect of antiretroviral drugs. *HIV Medicine*, *15*(4), 213–223. doi:10.1111/hiv.12104

Harezlak, J., Buchthal, S., Taylor, M., Schifitto, G., Zhong, J., Daar, E., ...HIV Neuroimaging Consortium. (2011). Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment. *AIDS*, *25*(5), 625–633. doi:10.1097/QAD.0b013e3283427da7

Haxby, J., Hoffman, E., & Gobbini, M. (2000). The distributed human neural system for face perception. *Trends in Cognitive Sciences*, *4*(6), 223–233.

Heaton, R. K., Clifford, D. B., Franklin, D. R., Woods, S. P., Ake, C., Vaida, F., ...CHARTER Group. (2010). HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology*, *75*(23), 2087–2096. doi:10.1212/WNL.0b013e318200d727

Heaton, R. K., Franklin, D. R., Deutsch, R., Letendre, S., Ellis, R. J., Casaletto, K., …CHARTER Group. (2015). Neurocognitive change in the era of HIV combination antiretroviral therapy: The longitudinal CHARTER Study. *Clinical Infectious Diseases*, *60*(3), 473–480. doi:10.1093/cid/ciu862

Heaton, R. K., Franklin, D. R., Ellis, R. J., McCutchan, J. A., Letendre, S. L., LeBlanc, S., … HNRC Group. (2011). HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. *Journal of NeuroVirology*, *17*(1), 3–16. doi:10.1007/s13365-010-0006-1

Heaton, R. K., Grant, I., Butters, N., White, D. A., Kirson, D., Atkinson, J. H., … HNRC Group. (1995). The HNRC 500--neuropsychology of HIV infection at different disease stages. HIV Neurobehavioral Research Center. *Journal of the International Neuropsychological Society*, *1*(3), 231–251.

Hestad, K., McArthur, J. H., Dal Pan, G. J., Selnes, O. A., Nance-Sproson, T. E., Aylward, E., …McArthur, J.C. (1993). Regional brain atrophy in HIV-1 infection: association with specific neuropsychological test performance. *Acta Neurologica Scandinavica*, *88*(2), 112–118.

Hoare, J., Fouche, J.-P., Spottiswoode, B., Joska, J. A., Schoeman, R., Stein, D. J., & Carey, P. D. (2010). White matter correlates of apathy in HIV-positive subjects: a diffusion tensor imaging study. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *22*(3), 313–320. doi:10.1176/appi.neuropsych.22.3.313

Howell, D. (2002). *Statistical methods for psychology* (5 ed.). Pacific Grove, CA: Duxbury Press.

Hull, M. W., & Montaner, J. S. G. (2013). HIV treatment as prevention: The key to an AIDS-free generation. *Journal of Food and Drug Analysis*, *21*(4), S95–S101. doi:10.1016/j.jfda.2013.09.043

INSIGHT START Study Group, Lundgren, J. D., Babiker, A. G., Gordin, F., Emery, S., Grund, B., …Neaton, J.D. (2015). Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. *The New England Journal of Medicine*, *373*(9), 795–807. doi:10.1056/NEJMoa1506816

Jernigan, T. L., Archibald, S. L., Fennema-Notestine, C., Taylor, M. J., Theilmann, R. J., Julaton, M. D …CHARTER Group. (2011). Clinical factors related to brain structure in HIV: the CHARTER study. *Journal of NeuroVirology*, *17*(3), 248–257. doi:10.1007/s13365-011-0032-7

Joassin, F., Pesenti, M., Maurage, P., Verreckt, E., Bruyer, R., & Campanella, S. (2010). Cross-modal interactions between human faces and voices involved in person recognition. *Cortex*, 47(3), 367-76. doi:10.1016/j.cortex.2010.03.003

Kallianpur, K. J., Shikuma, C., Kirk, G. R., Shiramizu, B., Valcour, V., Chow, D., ...Sailasuta, N. (2013). Peripheral blood HIV DNA is associated with atrophy of cerebellar and subcortical gray matter. *Neurology*, *80*(19), 1792–1799. doi:10.1212/WNL.0b013e318291903f

Kamat, R., Brown, G. G., Bolden, K., Fennema-Notestein, C., Archibald, S., Marcotte, T. D., …TMARC Group. (2014). Apathy is associated with white matter abnormalities in anterior, medial brain regions in persons with HIV infection. *Journal of Clinical and Experimental Neuropsychology*, *36*(8), 854–866. doi:10.1080/13803395.2014.950636

Kamat, R., Cattie, J. E., Marcotte, T. D., Woods, S. P., Franklin, D. R., Corkran, S. H., …Heaton, R.K. (2015). Incident major depressive episodes increase the severity and risk of apathy in HIV infection. *Journal of Affective Disorders*, *175*, 475–480. doi:10.1016/j.jad.2015.01.010

Katlama, C., Valantin, M. A., Algarte-Genin, M., Duvivier, C., Lambert-Niclot, S., Girard, P. M., ...Flandre, P. (2010). Efficacy of darunavir/ritonavir maintenance monotherapy in patients with HIV-1 viral suppression: a randomized open-label, noninferiority trial, MONOI-ANRS 136. *AIDS*, 24(15), 2365-74. doi:10.1097/QAD.0b013e32833dec20

Kirwan, C. B., & Stark, C. E. L. (2004). Medial temporal lobe activation during encoding and retrieval of novel face-name pairs. *Hippocampus*, *14*(7), 919–930. doi:10.1002/hipo.20014

Kolb, B., & Whishaw, I. Q. (2003). *Fundamentals of Human Neuropsychology* (5 ed.). New York: Worth Publisher.

Kopelman, M. D., & Stanhope, N. (1998). Recall and recognition memory in patients with focal frontal, temporal lobe and diencephalic lesions. *Neuropsychologia*, *36*(8), 785–795.

Kreitewolf, J., Friederici, A. D., & von Kriegstein, K. (2014). Hemispheric lateralization of linguistic prosody recognition in comparison to speech and speaker recognition. *NeuroImage*, *102*(2), 332–344. doi:10.1016/j.neuroimage.2014.07.038

Kuper, M., Rabe, K., Esser, S., Gizewski, E. R., Husstedt, I. W., Maschke, M., & Obermann, M. (2011). Structural gray and white matter changes in patients with HIV. *Journal of Neurology*, *258*(6), 1066–1075. doi:10.1007/s00415-010-5883-y

Lane, T. A., Moore, D. M., Batchelor, J., Brew, B. J., & Cysique, L. A. (2012). Facial emotional processing in HIV infection: Relation to neurocognitive and neuropsychiatric status. *Neuropsychology*, *26*(6), 713–722. doi:10.1037/a0029964

Letendre, S. (2008). Validation of the CNS Penetration-Effectiveness rank for quantifying antiretroviral penetration into the central nervous system. *Archives of Neurology*, *65*(1), 65–70. doi:10.1001/archneurol.2007.31

Letendre, S. (2011). Central nervous system complications in HIV disease: HIV-associated neurocognitive disorder. *Topics in Antiviral Medicine*, *19*(4), 137–142.

Letendre, S. L., FitzSimons, C., Ellis, R. J., Clifford, D., Collier, A. C., Gelman, B., …CHARTER Group. (2010). Correlates of CSF viral loads in 1221 volunteers of the CHARTER cohort. Presented at the 17th Conference on Retroviruses and Opportunistic Infections. February 16-19, 2010, San Francisco.

Levy, R. (2012). Apathy: A pathology of goal-directed behaviour. A new concept of the clinic and pathophysiology of apathy. *Revue Neurologique*, *168*(8-9), 585–597. doi:10.1016/j.neurol.2012.05.003

Levy, R. M., Bredesen, D. E., & Rosenblum, M. L. (1985). Neurological manifestations of the acquired immunodeficiency syndrome (AIDS): experience at UCSF and review of the literature. *Journal of Neurosurgery*, *62*(4), 475–495. doi:10.3171/jns.1985.62.4.0475

Levy, R., & Dubois, B. (2006). Apathy and the functional anatomy of the prefrontal cortex-basal ganglia circuits. *Cerebral Cortex*, *16*(7), 916–928. doi:10.1093/cercor/bhj043

Mandell, G. L. (2009). *Mandell, Douglas, and Bennett's. Principles and practice of infectious diseases* (7ed.). Edinburgh: Churchil Livingstone.

Manly, J. J., Touradji, P., Tang, M. X., & Stern, Y. (2003). Literacy and memory decline among ethnically diverse elders. *Journal of Clinical and Experimental Neuropsychology*, *25*(5), 680–690. doi:10.1076/jcen.25.5.680.14579

Marra, C. M., Zhao, Y., Clifford, D. B., Letendre, S., Evans, S., Henry, K., … AIDS Clinical Trials Group 736 Study Team. (2009). Impact of combination antiretroviral therapy on cerebrospinal fluid HIV RNA and neurocognitive performance. *AIDS*, *23*(11), 1359–1366. doi:10.1097/QAD.0b013e32832c4152

McArthur, J. C., Hoover, D. R., Bacellar, H., Miller, E. N., Cohen, B. A., Becker, J. T., ...Saah, A. (1993). Dementia in AIDS patients: incidence and risk factors. Multicenter AIDS Cohort Study. *Neurology*, *43*(11), 2245–2252.

McIntosh, R. C., Ironson, G., Antoni, M., Kumar, M., Fletcher, M. A., & Schneiderman, N. (2014). Alexithymia is linked to neurocognitive, psychological, neuroendocrine, and immune dysfunction in persons living with HIV. *Brain, Behavior, and Immunity*, *36*, 165–175. doi:10.1016/j.bbi.2013.10.024

McIntosh, R. C., Rosselli, M., Uddin, L. Q., & Antoni, M. (2015). Neuropathological sequelae of Human Immunodeficiency Virus and apathy: A review of neuropsychological and neuroimaging studies. *Neuroscience and Biobehavioral Reviews*, *55*, 147–164. doi:10.1016/j.neubiorev.2015.04.008

Mellors, J. W., Munoz, A., Giorgi, J. V., Margolick, J. B., Tassoni, C. J., Gupta, P., …Rinaldo, C.R. Jr. (1997). Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Annals of Internal Medicine*, *126*(12), 946–954.

Milders, M., Ietswaart, M., & Crawford, J. R. (2008). Social behavior following traumatic brain injury and its association with emotion recognition, understanding of intentions, and cognitive flexibility. *Journal of the International Neuropsychological Soc*iety, 14(2), 318-26. doi:10.1017/S1355617708080351

Miller, V., Mocroft, A., Reiss, P., Katlama, C., Papadopoulos, A. I., Katzenstein, T., … Lundgren, J.D. (1999). Relations among CD4 lymphocyte count nadir, antiretroviral therapy, and HIV-1 disease progression: results from the EuroSIDA study. *Annals of Internal Medicine*, *130*(7), 570–577.

Mohamed, M. A., Barker, P. B., Skolasky, R. L., Selnes, O. A., Moxley, R. T., Pomper, M. G., & Sacktor, N. C. (2010). Brain metabolism and cognitive impairment in HIV infection: a 3-T magnetic resonance

spectroscopy study. *Magnetic Resonance Imaging*, *28*(9), 1251–1257. doi:10.1016/j.mri.2010.06.007

Mollan, K. R., Smurzynski, M., Eron, J. J., Daar, E. S., Campbell, T. B., Sax, P. E., …Tierney C. (2014). Association between efavirenz as initial therapy for HIV-1 infection and increased risk for suicidal ideation or attempted or completed suicide. *Annals of Internal Medicine*, *161*(1), 1–10. doi:10.7326/M14-0293

Morales, M., Campo, P., Fernández, A., Moreno, D., Yáñez, J., & Sañudo, I. (2010). Normative data for a six-trial administration of a spanish version of the verbal selective reminding test. *Archives of Clinical Neuropsychology, 25*(8), 745–761. doi:10.1093/arclin/acq076

Moss, A. R., & Bacchetti, P. (1989). Natural history of HIV infection. *AIDS*, *3*(2), 55.

Muñoz-Moreno, J. A., Fumaz, C. R., Ferrer, M. J., Prats, A., Negredo, E., Garolera, M., …Clotet, B. (2008). Nadir CD4 cell count predicts neurocognitive impairment in HIV-infected patients. *AIDS Research and Human Retroviruses*, *24*(10), 1301–1307. doi:10.1089/aid.2007.0310

Navia, B. A., Cho, E. S., Petito, C. K., & Price, R. W. (1986). The AIDS dementia complex: II. Neuropathology. *Annals of Neurology*, *19*(6), 525–535. doi:10.1002/ana.410190603

Oddershede, L., Walker, S., Paton, N., & Stöhr, W. (2014). Cost-effectiveness analysis of protease inhibitor monotherapy vs. ongoing triple-therapy in the long-term management of HIV patients. *Journal of the International Neuropsychological Society,* 17(4 (Suppl 3)), 19498. doi:10.7448/IAS.17.4.19498

Panel on Antiretroviral Guidelines for Adults and Adolescents. (2009). Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Retrieved September 2, 2015, from http://aidsinfo.nih.gov/ContentFiles/Adultand AdolescentGL.pdf.

Paton, N. I., Stöhr, W., Arenas-Pinto, A., Fisher, M., Williams, I., Johnson, M., ...Protease Inhibitor Monotherapy Versus Ongoing Triple Therapy (PIVOT) Trial Team. (2015). Protease inhibitor monotherapy for long-term management of HIV infection: a randomised, controlled, open-label, non-inferiority trial. The Lancet HIV, 2(10), e417–26. doi:10.1016/S2352-3018(15)00176-9

Paul, R. H., Brickman, A. M., Navia, B., Hinkin, C., Malloy, P. F., Jefferson, A. L., … Flanigan, T.P. (2005a). Apathy is associated with volume of the nucleus accumbens in patients infected with HIV. *Journal of Neuropsychiatry*, *17*(2), 167–171. doi:10.1176/appi.neuropsych.17.2.167

Paul, R. H., Yiannoutsos, C. T., Miller, E. N., Chang, L., Marra, C. M., Schifitto, G., ...Navia, B.A. (2007). Proton MRS and neuropsychological correlates in AIDS dementia complex: evidence of subcortical specificity. *Journal of Neuropsychiatry*, *19*(3), 283–292. doi:10.1176/appi.neuropsych.19.3.283

Paul, R., Flanigan, T. P., & Tashima, K. (2005b). Apathy correlates with cognitive function but not CD4 status in patients with human immunodeficiency virus. *Journal of Neuropsychiatry and Clinical Neurosciences*, 17(1), 114-118. doi:10.1176/appi.neuropsych.17.1.114

Pence, B. W., Miller, W. C., Whetten, K., Eron, J. J., & Gaynes, B. N. (2006). Prevalence of DSM-IV-Defined Mood, Anxiety, and Substance Use Disorders in an HIV Clinic in the Southeastern United States. *Journal of Acquired Immune Deficiency Syndromes*, *42*(3), 298–306. doi:10.1097/01.qai.0000219773.82055.aa

Periáñez, J. A., Ríos-Lago, M., Rodríguez-Sánchez, J. M., Adrover-Roig, D., Sánchez-Cubillo, I., Crespo-Facorro, B., …Barceló, F. (2007). Trail Making Test in traumatic brain injury, schizophrenia, and normal ageing: sample comparisons and normative data. *Archives of Clinical Neuropsychology*, *22*(4), 433–447. doi:10.1016/j.acn.2007.01.022

Pérez-Valero, I., González-Baeza, A., Hernandez-Tamames, J.A., Monge, S., Arnalich, F., & Arribas, J.R. (2014a). Cerebral volumes, neuronal integrity and brain inflammation measured by MRI in patients receiving PI monotherapy or triple therapy. *Journal of the International AIDS Society*, *17*(4(Suppl 3)), 19578. doi:10.7448/IAS.17.4.19578

Pérez-Valero, I., González-Baeza, A., Estébanez, M., Monge, S., Montes-Ramírez, M. L., Bayón, C., … Arribas, J.R. (2014b). A prospective cohort study of neurocognitive function in aviremic HIV-infected patients treated with 1 or 3 antiretrovirals. *Clinical Infectious Diseases*, *59*(11), 1627–1634. doi:10.1093/cid/ciu640

Pérez-Valero, I., González-Baeza, A., Estébanez, M., Montes-Ramírez, M. L., Bayón, C., Pulido, F., ... Arribas, J.R. (2013). Neurocognitive Impairment in Patients Treated with Protease Inhibitor Monotherapy or Triple Drug Antiretroviral Therapy. *PLoS ONE*, *8*(7), e69493. doi:10.1371/journal.pone.0069493

Péron, J., Dondaine, T., Le Jeune, F., Grandjean, D., & Vérin, M. (2012). Emotional processing in Parkinson's disease: a systematic review. *Movement Disorders, 27*(2), 186–199. doi:10.1002/mds.24025

Pfefferbaum, A., Rosenbloom, M. J., Rohlfing, T., Kemper, C. A., Deresinski, S., & Sullivan, E. V. (2009). Frontostriatal fiber bundle compromise in HIV infection without dementia. *AIDS*, *23*(15), 1977–1985. doi:10.1097/QAD.0b013e32832e77fe

Pfefferbaum, A., Rosenbloom, M. J., Sassoon, S. A., Kemper, C. A., Deresinski, S., Rohlfing, T., & Sullivan, E. V. (2012). Regional Brain Structural Dysmorphology in Human Immunodeficiency Virus Infection: Effects of Acquired Immune Deficiency Syndrome, Alcoholism, and Age. *Biological Psychiatry*, *72*(5), 361–370. doi:10.1016/j.biopsych.2012.02.018

Philippi, C. L., Mehta, S., Grabowski, T., Adolphs, R., & Rudrauf, D. (2009). Damage to Association Fiber Tracts Impairs Recognition of the Facial Expression of Emotion. *Journal of Neuroscience*, *29*(48), 15089–15099. doi:10.1523/JNEUROSCI.0796-09.2009

Poropatich, K., & Sullivan, D. J. (2011). Human immunodeficiency virus type 1 long-term non-progressors: the viral, genetic and immunological basis for disease non-progression. *The Journal of General Virology*, *92*(2), 247–268. doi:10.1099/vir.0.027102-0.

Powderly, W. G.(2002). Sorting through confusing messages: the art of HAART. *Journal of Acquired Immune Deficiency Syndromes*, *31 (1)*, S3–9– discussion S24–5.

Pulido, F., Arribas, J. R., Delgado, R., Cabrero, E., González-García, J., Pérez-Elias, M. J., …OK04 Study Group. (2008). Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and two nucleosides for maintenance therapy of HIV. *AIDS*, *22*(2), F1–9. doi:10.1097/QAD.0b013e3282f4243b

Robertson, K. R., Su, Z., Margolis, D. M., Krambrink, A., Havlir, D. V., Evans, S., ...A5170 Study Team. (2010). Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. *Neurology*, *74*(16), 1260–1266. doi:10.1212/WNL.0b013e3181d9ed09

Robertson, K., Bayon, C., Molina, J. M., & McNamara, P. (2014). Screening for neurocognitive impairment, depression, and anxiety in HIV-infected patients in Western Europe and Canada. *AIDS Care*, *26*(12), 1555-61.doi:10.1080/09540121.2014.936813

Robertson, K., Liner, J., & Meeker, R. B. (2012). Antiretroviral neurotoxicity. *Journal of NeuroVirology*, *18*(5), 388–399. doi:10.1007/s13365-012-0120-3

Rudebeck, S. R., Scholz, J., Millington, R., Rohenkohl, G., Johansen-Berg, H., & Lee, A. C. (2009). Fornix microstructure correlates with recollection but not familiarity memory. *The Journal of Neuroscience*, *29*(47), 14987–14992. doi:10.1523/JNEUROSCI.4707-09.2009

Sacktor, N. C., Lyles, R. H., Skolasky, R. L., Anderson, D. E., McArthur, J. C., McFarlane, G., …Miller, E.N. (1999). Combination antiretroviral therapy improves psychomotor speed performance in HIV-seropositive homosexual men. Multicenter AIDS Cohort Study (MACS). *Neurology*, *52*(8), 1640–1647. doi:10.1212/WNL.52.8.1640

Sacktor, N., McDermott, M. P., Marder, K., Schifitto, G., Selnes, O. A., McArthur, J. C., …Epstein L. (2002). HIV-associated cognitive impairment before and after the advent of combination therapy. *Journal of NeuroVirology*, *8*(2), 136–142. doi:10.1080/13550280290049615

Sacktor, N., Skolasky, R. L., Tarwater, P. M., McArthur, J. C., Selnes, O. A., Becker, J., …Multicenter AIDS Cohort Study (MACS). (2003). Response to systemic HIV viral load suppression correlates with psychomotor speed performance. *Neurology*, *61*(4), 567–569. doi:10.1212/01.WNL.0000076477.71313.6E

Sacktor, N., Skolasky, R., Selnes, O. A., Watters, M., Poff, P., Shiramizu, B., …Valcour V. (2007). Neuropsychological test profile differences between young and old human immunodeficiency virus-positive individuals. *Journal of NeuroVirology*, *13*(3), 203–209. doi:10.1080/13550280701258423

Sander, D., Grandjean, D., Pourtois, G., Schwartz, S., Seghier, M. L., Scherer, K. R., & Vuilleumier, P. (2005). Emotion and attention interactions in social cognition: Brain regions involved in processing anger prosody. *NeuroImage*, *28*(4), 848–858. doi:10.1016/j.neuroimage.2005.06.023

Santos, J. R., Muñoz-Moreno, J. A., Moltó, J., Prats, A., Curran, A., Domingo, P., …Clotet, B. (2013). Virological efficacy in cerebrospinal fluid and neurocognitive status in patients with long-term monotherapy based on lopinavir/ritonavir: an exploratory study. *PLoS ONE*, *8*(7), e70201. doi:10.1371/journal.pone.0070201

Schirmer, A., & Kotz, S. A. (2006). Beyond the right hemisphere: brain mechanisms mediating vocal emotional processing. *Trends in Cognitive Sciences*, *10*(1), 24–30. doi:10.1016/j.tics.2005.11.009

Schlaepfer, T. E., Harris, G. J., Tien, A. Y., Peng, L., Lee, S., & Pearlson, G. D. (1995). Structural differences in the cerebral cortex of healthy female and male subjects: a magnetic resonance imaging study. *Psychiatry Research*, *61*(3), 129–135.

Sexton, C. E., Mackay, C. E., Lonie, J. A., Bastin, M. E., Terrière, E., O'Carroll, R. E., & Ebmeier, K. P. (2010). MRI correlates of episodic memory in Alzheimer's disease, mild cognitive impairment, and healthy aging. *Psychiatry Research*, *184*(1), 57–62. doi:10.1016/j.pscychresns.2010.07.005

Shapiro, M. E., Mahoney, J. R., Zingman, B. S., Pogge, D. L., & Verghese, J. (2013). Apathy correlates with cognitive performance, functional disability, and HIV RNA plasma levels in HIV-positive individuals. *Journal of Clinical and Experimental Neuropsychology*, *35*(9), 934–945. doi:10.1080/13803395.2013.838941

Spudich, S. S., & Ances, B. M. (2015). CROI 2015: Neurologic Complications of HIV Infection. *Topics in Antiviral Medicine*, *23*(1), 47–55.

Stern, Y., McDermott, M. P., Albert, S., Palumbo, D., Selnes, O. A., McArthur, J., …Dana Consortium on the Therapy of HIV-Dementia and Related Cognitive Disorders. (2001). Factors associated with incident human immunodeficiency virus-dementia. *Archives of Neurology*, *58*(3), 473–479.

Tam, J. W., & Schmitter-Edgecombe, M. (2013). The Role of Processing Speed in the Brief Visuospatial Memory Test – Revised. *The Clinical Neuropsychologist*, *27*(6), 962–972. doi:10.1080/13854046.2013.797500

The Centers for Disease Control. (1993). From the Centers for Disease Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *JAMA*, *269*(6), 729–730.

Theill, N., Martin, M., Schumacher, V., Bridenbaugh, S. A., & Kressig, R. W. (2011). Simultaneously measuring gait and cognitive performance in cognitively healthy and cognitively impaired older adults: the Basel motor-cognition dual-task paradigm. *Journal of the American Geriatrics Society*, *59*(6), 1012–1018. doi:10.1111/j.1532-5415.2011.03429.x

Thompson, M. A., Aberg, J. A., Hoy, J. F., Telenti, A., Benson, C., Cahn, P., …Volberding, P.A. (2012). Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA panel. *JAMA*, *308*(4), 387–402. doi:10.1001/jama.2012.7961

Thompson, P. M., Dutton, R. A., Hayashi, K. M., Toga, A. W., Lopez, O. L., Aizenstein, H. J., & Becker, J. T. (2005). Thinning of the cerebral cortex visualized in HIV/AIDS reflects CD4+ T lymphocyte decline. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(43), 15647–15652. doi:10.1073/pnas.0502548102

Tombaugh, T. N., Kozak, J., & Rees, L. (1999). Normative data stratified by age and education for two measures of verbal fluency: FAS and animal naming. *Archives of Clinical Neuropsychology*, *14*(2), 167–177.

Trilla, A., Mensa, J., & Gatell, J. M. (1988). Infeccion por el Virus de la Inmunodeficiencia Humana y SIDA. In C. Rozman, *Medicina Interna* (pp. 2341–2352). Barcelona: Doyma.

Trites, R. L. (1977). *Neuropsychological Test Manual*. Ottawa, Ontario, Canada: Royal Ottawa Hospital.

UNAIDS. (2013). *Global Report: UNAIDS report on the global AIDS epidemic 2013*. Geneva: UNAIDS.

Valcour, V., Shikuma, C., Shiramizu, B., Watters, M., Poff, P., Selnes, O., … Sacktor N. (2004). Higher frequency of dementia in older HIV-1 individuals: the Hawaii Aging with HIV-1 Cohort. *Neurology*, *63*(5), 822–827.

Valcour, V., Sithinamsuwan, P., Letendre, S., & Ances, B. (2010). Pathogenesis of HIV in the central nervous system. *Current HIV/AIDS Reports*, *8*(1), 54–61. doi:10.1007/s11904-010-0070-4

Van Duijn, E., Craufurd, D., Hubers, A. A. M., Giltay, E. J., Bonelli, R., Rickards, H., ...European Huntington's Disease Network Behavioural Phenotype Working Group. (2014). Neuropsychiatric symptoms in a European Huntington's disease cohort (REGISTRY). *Journal of Neurology, Neurosurgery, and Psychiatry*, *85*(12), 1411–1418. doi:10.1136/jnnp-2013-307343

Vance, D. E., Fazeli, P. L., Grant, J. S., Slater, L. Z., & Raper, J. L. (2013). The Role of neuroplasticity and cognitive reserve in aging with HIV. *Journal of Neuroscience Nursing*, *45*(5), 306–316. doi:10.1097/JNN.0b013e31829d8b29

Wang, M., Wang, Q., Ding, H., & Shang, H. (2015). Association of Hippocampal Magnetic Resonance Imaging with Learning and Memory Deficits in HIV-1 Seropositive Patients. *Journal of Acquired Immune Deficiency* Syndromes. Advance online publication. doi:10.1097/QAI.0000000000000789

Wechsler, D. (1997). *WAIS-III, Wechsler adult intelligence scale: Administration and scoring manual* (3rd ed.). San Antonio:The Psychological Corporation.

White, D. A., Heaton, R. K., & Monsch, A. U. (1995). Neuropsychological studies of asymptomatic human immunodeficiency virus-type-1 infected individuals. The HNRC Group. HIV Neurobehavioral Research Center. *Journal of the International Neuropsychological Society*, *1*(3), 304–315.

White, W., Grant, J. S., Pryor, E. R., Keltner, N. L., Vance, D. E., & Raper, J. L. (2012). Do social support, stigma, and social problem-solving skills predict depressive symptoms in people living with HIV? A mediation analysis. *Research and Theory for Nursing Practice*, *26*(3), 182–204.

Wildgruber, D., Hertrich, I., Riecker, A., Erb, M., Anders, S., Grodd, W., & Ackermann, H. (2004). Distinct frontal regions subserve evaluation of linguistic and emotional aspects of speech intonation. *Cerebral Cortex*, *14*(12), 1384–1389. doi:10.1093/cercor/bhh099

Winston, A., Arenas-Pinto, A., Stöhr, W., & Fisher, M. (2013). Neurocognitive function in HIV infected patients on antiretroviral therapy, 8(4), e61949. doi:10.1371/journal.pone.0061949

Witteman, J., van Ijzendoorn, M. H., van de Velde, D., van Heuven, V. J. J. P., & Schiller, N. O. (2011). The nature of hemispheric specialization for linguistic and emotional prosodic perception: A meta-analysis of the lesion literature. *Neuropsychologia*, *49*(13), 3722–3738. doi:10.1016/j.neuropsychologia.2011.09.028

Yonelinas, A. P. (2013). The hippocampus supports high-resolution binding in the service of perception, working memory and long-term memory. *Behavioural Brain Research*, *254*, 34–44. doi:10.1016/j.bbr.2013.05.030

8. ANNEXES

8.1. Scientific communications related to this doctoral thesis

8.1.1.Presentations at scientific meetings

INTERNATIONAL CONGRESS

14th European AIDS Conference. Belgrade 2013

A. González-Baeza, C. Bayón, I. Pérez-Valero, M. Estébanez, S. Monge, M. Lagarde, F. Pulido, J.I. Bernardino, F.X. Zamora, M. Montes, JJ González-García, J.R. Arribas. *Is there a difference in the neuropsychological profile of HIV-infected patients on protease inhibitor monotherapy vs triple therapy?*. **Poster presentation.**

12th International Congress on Drug Therapy in HIV Infection. Glasgow 2014

Gonzalez-Baeza, A ; Perez-Valero, I ; Carvajal-Molina, F ; Bayon, C ; Montes-Ramirez, M ; Bernardino, JI ; Arribas, JR. *Facial emotional processing deficits in long-term HIV-suppressed patients.* **Poster presentation.**

15th European AIDS Conference. Barcelona 2015

Gonzalez-Baeza, A; Arribas, JR; Perez-Valero, I; Monge, S; Bayón, C; Carvajal, F. Brain-related *Voice Emotion Processing Deficits in HIV-infected Patients.* **Poster presentation.**

8.1.2. Scientific publications

PUBLISHED PAPER

González-Baeza A, Carvajal F, Bayón C, Pérez-Valero I, Estébanez M, Bernardino JI, Monge S, Lagarde M, Hernando A, Arnalich F, Arribas JR. *Pattern of neurocognitive function in patients receiving boosted protease inhibitor monotherapy: a detailed neuropsychological study*. J Neurovirol. 2014 Aug;20(4):362-70. doi: 10.1007/s13365-014-0251-9.

PAPER CURRENTLY UNDER REVIEW

González-Baeza A, Carvajal F, Bayón C, Pérez-Valero I, Montes-Ramírez M, Arribas JR. *Facial emotion processing in aviremic HIV-infected adults.*

PAPER CURRENTLY UNDER PREPARATION

González-Baeza A, José R Arribas, Ignacio Pérez-Valero, Susana Monge, Carmen Bayón, Pilar Martín, Sandra Rubio, Fernando Carvajal*. Impaired emotional prosody processing in HIV-infected individuals.*