The Role of Hemispheric Lateralisation in Immunity & Human Immunodeficiency Virus Type 1 (HIV-1)

A thesis submitted for the degree of
Doctor of Philosophy

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ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANS	Autonomic Nervous System
ART	Antiretroviral Therapy
ARV	Antiretroviral
BBB	Blood-Brain Barrier
CDC	(US) Centres for Disease Control
CMV	Cytomegalovirus
CNS	Central Nervous System
Con-A	Concanavalin-A
CRF	Circulating Recombinant Form
CRI	Co-Receptor Inhibitor
CRP	C-Reactive Protein
CT	Computer Tomography
CVO	Circumventricular Organs
DA	Dopamine
DC	Dendritic Cells
DNA	Deoxyribonucleic Acid
DTH	Delayed-Type Hypersensitivity
EEG	Electroencephalogram
FIs	Fusion Inhibitors
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
HAART	Highly Active Antiretroviral Therapy
HADS	Hospital Anxiety and Depression Scale
HEPS	Highly Exposed Persistently Seronegative
HIV	Human Immunodeficiency Virus
HIV+	HIV Seropositive
HL	Hemispheric Lateralisation
HLA	Human Leukocyte Antigen
HPA	Hypothalamic-Pituitary-Adrenal
HPT	Hemispheric Preference Test
HSV	Herpes Simplex Virus
IDU	Injection Drug Users
IFN	Interferon
IHI	Interhemispheric Inhibition
IL	Interleukin
INIs	Integrase Inhibitors
IQ	Intelligence Quotient
LBT	Line Bisection Test
LHL	Left Hemispheric Lateralisation
LTNPs	Long-Term Non Progressors
M-CSF	Macrophage Colony-Stimulating Factor
MDR	Multi Drug Resistant
MIP	Macrophage Inflammatory Protein
MMSE	Mini Mental State Examination
MR	Multiple Regression
mRNA	Messenger Ribonucleic Acid
MSM	Men who have Sex with Men
NK Cell	Natural Killer Cell

NKCA	Natural Killer Cell Activity
NNRTIs	Non-Nucleoside Reverse-Transcriptase Inhibitors
NP	Neuropeptide
NRTIs	Nucleoside Reverse-Transcriptase Inhibitors
NT	Neurotransmitter
NtRTIs	Nucleotide Reverse-Transcriptase Inhibitors
PFC	PreFrontal Cortex
PHA	Phytohemagglutanin
PHI	Primary HIV Infection
PIs	Protease Inhibitors
PNS	Parasympathetic Nervous System
POMS	Profile of Mood States
PrEP	Pre-Exposure Prophylaxis
PTSD	Post-Traumatic Stress Disorder
PW	PokeWeed
R4	CXCR4-tropic
R4R5	CXCR4/CCR5-tropic
R5	CCR5-tropic
RNA	Ribonucleic Acid
RT	Reverse-Transcriptase
rTMS	Repetitive Transcranial Magnetic Stimulation
SCF	Stem Cell Factor
SD	Standard Deviation
SES	Socio-economic Status
SHI	Symptomatic HIV Infection
S-IgA	Salivary Immunoglobulin A
SIV	Simian Immunodeficiency Virus
SNS	Sympathetic Nervous System
SP	Substance P
SPSS	Statistical Package for Social Sciences
SSA	Sub-Saharan Africa
STI	Sexually Transmitted Infection
TB	Tuberculosis
TeMS	Transcranial-Electromagnetic Stimulation
TNF	Tumour Necrosis Factor
TPO	Temporo-Parieto-Occipital (cortex)
UNAIDS	Joint United Nations Programme on HIV/AIDS
URF	Unique Recombinant Forms
UZB	Universiteit Ziekenhuis Brussel
VL	Viral Load
WBC	White Blood Cells
WHO	World Health Organisation

ABSTRACT

Neuromodulation of the immune system has been described to be influenced by hemispheric lateralisation (HL), the stable tendency to relatively utilise one hemisphere or its functions over another. To date there has not been a systematic review of research in this phenomenon conducted, and only one study has examined the effects of HL on the progression of a disease – Human Immunodeficiency Virus (HIV). That research was conducted on a small sample with little control for confounders. The present work sought to compile a systematic review of literature concerning HL and immunity in humans, using effect size analysis. Further, the present work also describes an empirical advancement of this earlier HIV study with stricter control over confounds in a larger sample. The findings corroborated the theory of asymmetrical immune influence by HL via the systematic review showing clear, relatively consistent and strong relationships between left-HL and immunopotentiation. The empirical prospective study extended current knowledge of this relationship in HIV to identify a moderator – HAART treatment. Specifically, left-HL predicted better immunity in HIV-1 patients independent of confounders, with further findings of the same pattern in untreated patients, but not in HAART-treated patients. Further observations were made between HL and HIV-relevant behaviours, again adding to current knowledge. The finding of left-HL being associated with fewer sexual partners in Europeans presents new information of relevance to public health. The combined findings of the present work suggest that left-HL has predictive value in illness (HIV-1) and in general immunity. The present work adds to the existing knowledge new information concerning a moderating factor of the HL-immunity relationship in HIV, and behavioural implications of HL which impact upon HIV disease. Potential explanations for moderation, proposals for neurobiological mechanisms and direction towards future, more rigourous study in the field, both in HIV and immunity, are discussed.

The Role of Hemispheric Lateralisation in Immunity and Human Immunodeficiency Virus Type 1

Introduction

PSYCHONEUROIMMUNOLOGY AND THE BIDIRECTIONAL MANNER OF COMMUNICATION BETWEEN THE BRAIN AND IMMUNE SYSTEM

Psychoneuroimmunology describes a bi-directional manner of communication between the central nervous system (CNS) and immune system in humans and other animals (Bellinger *et al*., 2008; Ferone *et al.*, 2006; Webster *et al*., 2002) and how psychological factors influence immunity. The composite mechanisms of this bi-directional communication rely on networks associated with neurological, endocrine and immune systems (Banks, 2004; Besedovsky & del Ray, 1996; 2007; Butts & Sternberg, 2008; Taub, 2008). The immune system communicates with the CNS once an infection has been encountered in the periphery to initiate a sequence of events termed *sickness behaviour* which serve to provide optimum behavioural conditions to facilitate immune defence and recovery (Hopkins, 2007; Konsman *et al*., 2002; Rivest, 2003; Vollmer-Conna *et al.* 2004). The CNS communication with the immune system largely comes in the form of modulating immune parameters, in ways which are still being discovered, including sympathetic and neuroendocrine pathways (Ferone *et al.*, 2006; Neveu, 1988; Webster *et al.*, 2002; Wrona, 2006). Evidence of cerebral influence on immune function also comes from neurophysiological observations concerning hemispheric lateralisation (HL), a phenomenon still under research which not only demonstrates CNS to immune system communication but also serves to explain some individual differences within this association (Neveu, 1988; 1991; 1992).

IMMUNE TO BRAIN COMMUNICATION

Perhaps the most obvious illustration of the influence of the immune system on the CNS is the generation of sickness behaviour (Banks, 2004; Besedovsky & del Ray, 1996, 2007; Konsman *et al*., 2002). Sickness behaviour is a collective term for behavioural responses to the increased circulation of proinflammatory cytokines resulting from peripheral illness, including somnolence, hyperalgesia, lethargy and anorexia, which facilitate convalescence (Banks, 2004; Hopkins, 2007; Rivest, 2003). On a biological level, there is further evidence for immune system influence on CNS functioning, most notably in stimulation of the hypothalamo-pituitary adrenal (HPA)

axis (in releasing corticotropin releasing hormone and vasopressin) by interleukin-(IL)1, IL-2, IL-3, IL-6, IL-8, IL-11, IL-12, tumour necrosis factor- (TNF) α, interferon- (IFN) γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Goncharova & Tarakanov, 2007; Guyon, *et al.*, 2008; Hopkins, 2007). Transport of immune signals to the brain is largely considered to occur by three means; via the vagus nerve, the circumventricular organs (CVO) and through mediators on both sides of the blood brain barrier (BBB); which together make up the *Dorsal Vagal Complex* (Guyon *et al.*, 2008; Quan & Banks, 2007; Rivest, 2003; Wrona, 2006).

There is also evidence that immune cells can exude hormones, neuropeptides and neurotransmitters such as catecholamines, which are transmitted to the brain (either systemically or via neural pathways – most notably the vagus nerve) and ultimately create further neuroendocrine alterations (Banks, 2004; Besedovsky & Del Ray, 1996, 2007; Hopkins, 2007; Wrona, 2006). These observations have led to the immune system being characterised as another sensory organ used by the brain to ensure survival of the organism (Besedovsky & Del Ray, 1996, 2007; Goncharova & Tarakanov, 2007; Hopkins, 2007; Wrona, 2006).

Finally, there is evidence for centrally located receptors for immune signals and for cytokines such as IL-1(α and β), IL-2, IL-4, IL-6, TNF-α, IFN-γ, macrophage colony stimulating factor (M-CSF), and stem cell factor (SCF) (Besedovsky & Del Ray, 1996; Goncharova & Tarakanov, 2007; Hopkins, 2007; Rivest, 2003; Wrona, 2006), indicating that such immune signals operate in the CNS.

CENTRAL NERVOUS SYSTEM COMMUNICATION WITH THE IMMUNE SYSTEM

One of the first demonstrations of a neural influence on immune system function came in the form of a conditioning experiment by Ader & Cohen in 1975 (Ader, 2003; Ader & Cohen, 1975). They demonstrated an immune response to a drug or antigen could, by classical conditioning, become associated with a taste stimulus (Ader, 2003; Ader & Cohen, 1975). Since this discovery much research has been undertaken to elucidate the nature of this relationship and the mechanisms by which these influences take place. This very large field of research can be largely split into two groups based on the mechanism of communication; communication to organs and communication to immune cells (Banks, 2004; Bellinger *et al.*, 2006, 2008; Besedovsky & Del Ray, 1996; Butts & Sternberg, 2008; Ferone *et al.*, 2006).

CNS communication with immune organs

Organs of the lymphatic system, such as bone marrow, thymus, spleen, mucosal lymphoid tissues and lymph nodes, are innervated by autonomic fibres – mainly of the sympathetic division, but parasympathetic involvement has recently been described (Bellinger *et al.* 2006; 2008; Quan & Banks, 2007; Wrona 2006). The main regions of the brain which feed into these communication pathways are: the HPA axis, most notably the anterior, medial, lateral aspects and paraventricular nucleus of the hypothalamus; the CVO; certain limbic structures, most notably the amygdala and hippocampus; the cortex; the cerebellum; the dorsal vagal complex and the midbrain periaqueductal grey matter (Banks, 2004; Bellinger *et al*. 2006; 2008; Quan & Banks, 2007; Webster *et al.*, 2002; Wrona 2006). The purpose of these innervations ranges from direct regulation of function, such as haematopoiesis, lymphopoeisis and vasomotor function; following the provision of neurotransmitters (NT), neuropeptides (NP) and hormonal signalling (Banks, 2004; Bellinger *et al.* 2006; 2008; Quan & Banks, 2007; Wrona 2006).

CNS communication with immune cells

The main means of direct communication with immune cells comes in the form of neurochemical signalling, using NTs, NPs and hormones (Bellinger *et al.*, 2008; Besedovsky & Del Ray, 1996; 2007; Butts & Sternberg, 2008; Levite, 2008; Taub, 2008). Receptors for various hormones, NPs and NTs have been found to be expressed on the surface of many immune cell types, making a main "meeting point" for CNS-immune communication (Basu & Dasgupta, 2000; Ferone *et al.*, 2006; Levite, 2008; McKenna *et al.*, 2002; Wrona, 2006). These NPs, NTs and hormone receptors include, but are not limited to, receptors for: acetylcholine, β-adrenergic agents, dopamine, vasointestinal peptide, prolactin, growth hormone, glutamate, serotonin, corticosteroids, neuropeptide Y, somatostatin, cortistatin, estradiol, insulin, testosterone, endorphins, substance P, μ- δ- and κ-opioids and enkephalins, but are not equally expressed across all immune cells (Levite, 2008; Taub, 2008; Wrona, 2006).

DIFFERENTIAL CNS INFLUENCES ON THE IMMUNE SYSTEM: HEMISPHERIC LATERALISATION

The nature of the effects of the CNS on the immune system can be dependent upon many factors, including hemispheric lateralisation (HL); the stable tendency to relatively activate or utilise the functions of one hemisphere versus the other. The two hemispheres of the human brain have different functional specialisations, and it is well known that one of the hemispheres will be functionally dominant to the other (Cerqueira *et al*., 2008; Hugdahl, 2000; Neveu, 1988, 1991, 1992). The two hemispheres of the brain are known to act differentially upon behaviour, and mood, manifested in psychiatric and neurological disorders and act differentially on immunity (Coan & Allen, 2004; Davidson, 2003; Neveu, 1992). Experimental studies in animals across the last two decades have demonstrated that damage to the left or right hemispheres of the brain results in opposite immunological reactions (Neveu, 1988, 1991, 1992). Damage to the left hemisphere results in the depression of immunological parameters; such as T-lymphocyte proliferation, natural killer cell activity (NKCA), IL-2, and Immunoglobulin G antibody production (Neveu, 1988, 1991, 1992). Damage to the right hemisphere can produce either no immunological change, or even enhance certain immune parameters (Neveu, 1988, 1991, 1992).

In 1982, one research group developed a theory based on the association of prenatal testosterone exposure, what they termed “anomalous dominance” (i.e. abnormal language lateralisation, left handedness) (Geschwind & Behan, 1982). In observing higher incidences of left-handedness amongst populations of individuals with developmental and immune disorders, Geschwind and Behan hypothesised that this anomalous dominance was partly responsible for the variation observed across individuals in susceptibility to illnesses (Geschwind & Behan, 1982; Morfit & Weekes, 2001). This proposal was supported through large population surveys of left-handed individuals, along with population surveys of sinistrality in patients with migraine and immune disorders (Geschwind & Behan, 1982). Whilst this research was vital to the initiation of investigation of HL effects on immune function, it was fraught with conceptual and methodological flaws. Most notable of which was “anomalous dominance”, which cannot be determined by handedness alone, since also among right handed people alone, many have a right (and not left) stronger hemisphere activity, and “anomalous dominance” is too broad a concept in itself to be

used as a definition of cerebral dominance pattern (McManus & Bryden, 1991). Indeed, handedness is very poorly, if at all, correlated with HL, but rather may be indicative of language lateralisation only (Jung *et al.*, 2003; Toga & Thompson, 2003). Moreover, research with animals has suggested that "handedness" effects on the immune system can be abolished with unilateral cortical ablation to the left hemisphere (Neveu *et al.*, 1991), and may therefore not be a stable index of HL activation.

Studies of Lateralisation and Immunity: The present and the future

Since the landmark research of Geschwind and Behan (1982), more research groups have begun to examine the relationship between HL and immune function in humans. The emphasis of research in this field is now centred upon *activational* HL (i.e. the overall lateralisation of hemispheric activation) and on relative utility of certain functions associated with one hemisphere, rather than on the psychomotor concept of handedness (i.e. *specialisation*). With the developments in science which have occurred in the last two decades it is now possible to easily view individuals' brain activation patterns, assess neuropsychological functioning via multitudes of tests and perform sophisticated laboratory testing to analyse the components of blood and immunity. These developments have made the examination of the brain and immune relationships infinitely more feasible and accurate, yet more complex as well.

Whilst the study of the HL-immunity relationship has continued, there has not been a synthesised examination of the cumulative data. Considering data in this area has been generated for almost 30 years, an evaluation of the combined associations of the literature is both timely and important. Moreover, only one study to date has examined the implications of this relationship on the progression of a disease. This study explored the predictive capability of HL on the prognostic immune markers of T-cells in Human Immunodeficiency Virus (HIV) and provided support for the relationship between laterality and immunity (Gruzelier *et al.*, 1996). Further, this study provided support for the theory that HL has opposite effects on immunity, insomuch as left HL provides immunopotentiation and right HL elicits immunosuppression. These findings echo previous findings in both animal and human studies (Ivashkova *et al.*, 2002; Neveu, 1992), but – crucially - apply these findings to a disease. However, this study had several critical limitations, including lack of

statistical control for baseline immunity, a modest sample size and type, and nearly no methodological or statistical control for variables which create interference in the general functioning of the immune system (e.g. other illnesses such as cancer), or the modulation of HIV disease course (e.g. psychological morbidity, duration of illness). Therefore, despite the initial encouraging results of this study, the findings need to be replicated in a larger sample of HIV patients, with more advanced statistical and methodological control.

THESIS AIMS & OBJECTIVES

The role of HL in immunity

Given the wealth of literature available concerning the relationship between HL and immunity in humans there is a need for compilation and assessment of the data. As this is a relatively new field of research, it is important to evaluate the strengths of evidence and identify gaps in knowledge and future methodological requirements. Systematic reviews provide a synthesized appraisal of data to guide future research and best-practice in parallel investigations, and are vital to assess generalisability of existing findings (Cook *et al.*, 1997; Mulrow, 1994). Further, they are essential for the identification of methodological limitations inherent within that field, and can guide research to overcome these limitations. In order to substantially posit that HL impacts immunity, it is necessary to statistically evaluate the strength of the current evidence. If such a postulation can be corroborated by the combined findings of the available literature, this could provide a validation for this proposal in assessing the modulation of the immune system by the brain. Moreover, the implications of such a relationship could be applied to many areas of research; in identifying individual differences in disease course, potential targets for interventions and considerations in the treatment of disease.

It is therefore one of the aims of the present work to conduct a systematic review of available literature on humans. This review will summarise the data, statistically evaluate the strengths of the data through effect size analysis, and provide detailed quality analysis of the research methodological quality, with guidelines for future directions. By analysing the data in such a way, this provides a unique opportunity to establish and formulate a research advancement guided by the cumulative knowledge of studies thus far conducted.

The role of HL in HIV

To provide a practical application of the present theory, and to advance the currently available evidence in a disease model, a study was designed and executed to advance the findings of Gruzelier and colleagues. Inclusion of the Gruzelier *et al.* study in the aforementioned systematic review provides guidance on the methodological limitations and potential for expansion of the findings. Detailed exploration of literature concerning the biological aspects of HIV pathogenesis and those variables already known to influence disease course will be conducted in order to provide a thorough and considered approach to the research. Furthermore, the addition of exploring behavioural aspects of HL and HIV will add new data to the existing body of literature in these research areas. Moreover, analysis will be conducted to identify potential moderating factors in the relationship between HL and HIV immunity to help understand those factors which drive or facilitate the relationship.

Thus, the second objective of the present thesis was to conduct an investigation in to the predictive capabilities of HL in relation to the outcome of HIV-specific immunity. This was done using the basic framework from the Gruzelier study, with the addition of statistical and methodological control for those variables known to modulate HIV disease course, to increase inferential validity, and to allow the generalising of the findings to larger groups of HIV patients.

Chapter 1

A Biomedical Account of Human Immunodeficiency Virus

CLASSIFICATION

Human immunodeficiency virus (HIV) is of the genus *Lentivirus*, family *Retroviridae*, termed a retrovirus due to its ability to genetically reproduce with a reverse template (i.e., transcribing DNA from RNA) (Butler *et al.*, 2007; Klimas, *et al.*, 2008). It is suggested that HIV was zoonotically transferred from non-human primates (in the form of *Simian Immunodeficiency Virus*: SIV) to humans, where it has flourished and spread for over 30 years (Butler *et al.*, 2007; Lever, 2005; Reeves & Doms, 2002). HIV infiltrates the immune system of the host and proliferates, exhausting the host's immune system until the host reaches total immune failure and subsequently dies from infection or neoplasia (Graham, 1998; Sierra *et al.*,2005; Weber, 2001; Wilkinson & Gotch, 2001). The period of immune failure is known as Acquired Immune Deficiency Syndrome (AIDS) and is unequivocally related to HIV infection (Harris & Bolus, 2008; Levy, 2006).

Two genetically distinct species of HIV exist; HIV-1 and HIV-2; and both can lead to AIDS (Grant & De Cock, 2001; Levy, 2006; Lewthwaite & Wilkins, 2005; Reeves & Doms, 2002). The two types are pathogenically similar (although HIV-2 appears to be less virulent), but differ in terms of epidemiology (Grant & De Cock, 2001; Lever, 2005; Lewthwaite & Wilkins, 2005; Reeves & Doms, 2002). HIV-1 is now considered to be pandemic, whereas HIV-2 is relatively endemic; being broadly confined to the geographical regions of West Africa, southern Europe and in the Southwest area of India (Grant & De Cock, 2001; Levy, 2006; Reeves & Doms, 2002; Roquebert *et al.*, 2009). Due to its reduced virulence, confined areas of infection and similarity of medical treatment, HIV-2 receives less attention from the scientific community than HIV-1, which is responsible for many more new infections and AIDS-related deaths worldwide (Reeves & Doms, 2002).

HIV-1 is further subdivided in to strains (by nucleotide sequence), subtypes within the strains, and combinations of subtypes (circulating recombinant forms: CRF) each representing different pathogenic speeds and risk factors (Roquebert *et al.*, 2009; Spira *et al.*, 2003). HIV-1 is split in to three groups (strains); M (main group); O (outlier); and N (non-M, non-O) (Butler *et al.*, 2007; Klimas *et al.*, 2008; Roquebert *et al.*, 2009; Simon *et al.*,2006). Amongst group M there are 9 main subtypes (A, B, C, D, F, G, H, J and K), as well as many genetically distinct CRFs, derived from the combination of at least two of these subtypes (Klimas *et al.*, 2008; Roquebert *et al.*,

2009; Skar *et al.*,2011). At least 40 CRFs have been identified so far, with two emerging frequently in genotypic analyses worldwide: CRF01_AE and CRF02_AG (Skar *et al*, 2011). Within HIV-1, non-B subtypes represent 90% of circulating virus strains, with subtype C (most common in Southern Africa and Asia) being responsible for approximately 50% of global infections (Roquebert *et al*., 2009). Subtypes A and D are most commonly found in East Africa, CRF02_AG in West Africa, and subtype B in North America, Australia and Western Europe (Klimas *et al.*, 2008; Roquebert *et al.*, 2009).

EPIDEMIOLOGY

HIV/AIDS is one of the most frequent causes of death across the world, ranking 7th in the worldwide statistics, rising to 6th and 3rd in middle and low income countries respectively (WHO, 2011). Recent statistics from the Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organisation (WHO) epidemic update show that at the end of 2010 there were approximately 34 million people living with HIV across the world (UNAIDS, 2011). The estimates of new infections in that year are 2.7 million (UNAIDS, 2011), an average of over 7000 new infections every day. It is estimated that 1.8 million people died from AIDS in 2010 (UNAIDS, 2011), bringing a daily figure of nearly 5000 people.

Of these global statistics, the overwhelming majority of infections, deaths and numbers of people living with HIV are observed in the region of Sub-Saharan Africa (SSA; see Figure 1) (UNAIDS, 2011). The largest singular contribution of this epidemic in SSA, and indeed the global pandemic, comes from South Africa, which is now estimated to have 5.6 million people living with HIV/AIDS (UNAIDS, 2010). The estimated number of children living with HIV increased to 2.5 million in 2009; however the number of children being born with HIV is decreasing overall (UNAIDS, 2010). The geographical region (designated by UNAIDS) that seconds SSA in terms of people living with HIV is Asia, which stands at 4.8 million as compared to the 22.9 million in SSA (UNAIDS, 2011), which illustrates the relative severity of the situation in Africa. One country in SSA has a devastatingly high saturation of HIV infections; Swaziland now has a 25.9% adult prevalence (UNAIDS, 2010).

A recent upsurge of HIV infections in the region of Eastern Europe and Central Asia has seen the number of people living with the disease increase by over 250% since 2001 (UNAIDS, 2011). The two countries within this region that have the highest

prevalence rates (>1%) are the Russian Federation and Ukraine, which combined account for over 90% of new infections in the region (UNAIDS, 2010; 2011). The comparably affluent UNAIDS region of North America & Western and Central Europe was estimated to have 2.2 million people living with HIV/AIDS in 2010, an increase of 34% since 2001 (UNAIDS, 2011). The largest proportion of these infections are reported to be amongst men who have sex with men (MSM), with data from 23 European countries reporting an 86% rise in HIV diagnoses in this demographic between 2000 and 2006 (UNAIDS, 2010). In 2007 there were 3160 new diagnoses among MSM in the UK alone, the highest ever reported at that time (UNAIDS, 2010). New diagnoses have risen by more than 50% in the UK between 2000 and 2009 (UNAIDS, 2011). Similarly, new diagnoses have doubled in other European countries in that time; in Bulgaria, Czech Republic, Hungary, Lithuania, Slovakia and Slovenia (UNAIDS, 2011). Disparities within the countries in this global region abound, with reports of African-American women in the US thought to be 19 times more likely to contract HIV in comparison to corresponding Caucasian women (UNAIDS, 2010).

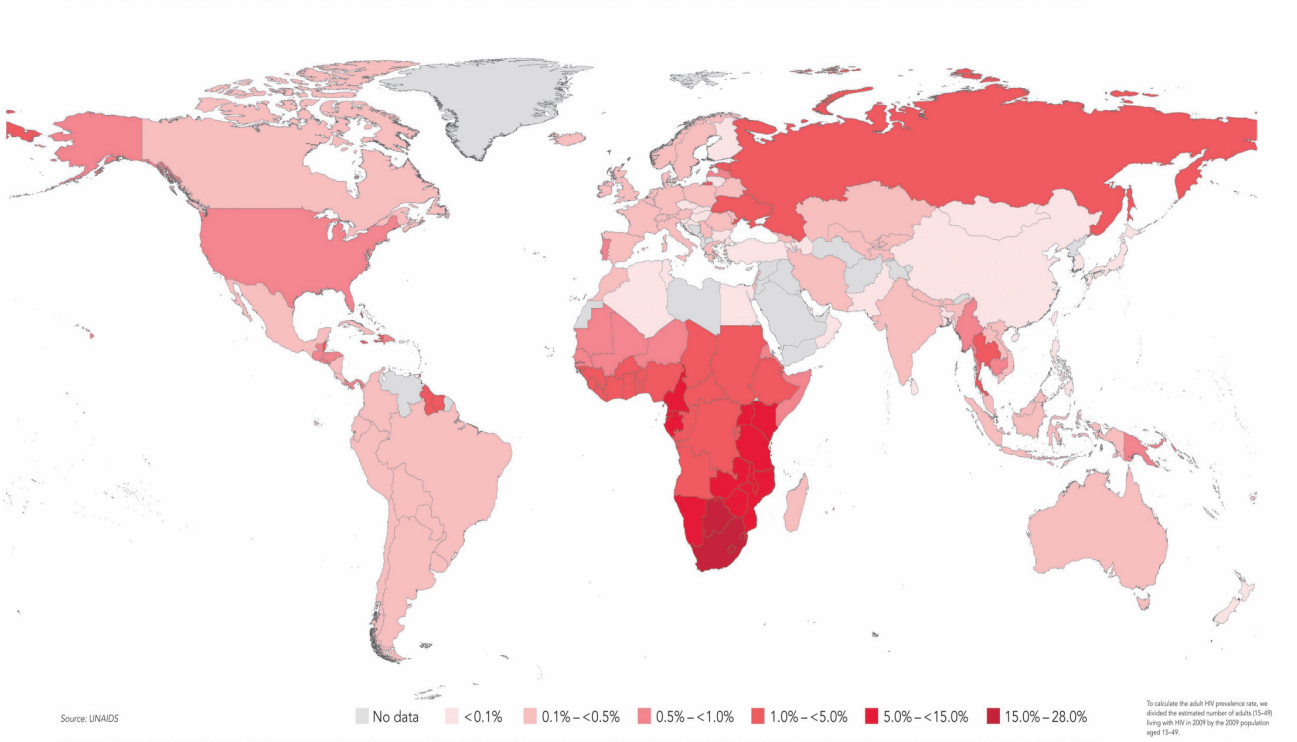

Figure 1. Global prevalence of HIV, 2009. (UNAIDS, 2010) The darker areas show highest concentration of prevalence (15-28%)

TRANSMISSION

When AIDS was first discovered in 1981 in the US, it was in a demographic of homosexual men and was considered, at that time, to be related to homosexual activity (Levy, 2006; WHO, 2007). It was another two years before HIV was discovered and implicated as the cause of AIDS (Levy, 2006; WHO, 2007). By 1984 a heterosexual epidemic of AIDS had been discovered across the Atlantic in Africa (WHO, 2007) suggesting that the disease was communicable beyond homosexual demographic groups. Today, the vast majority of new infections in the developing world are borne from heterosexual contact, seconded by vertical perinatal and breast-milk transmission (Grant & De Cock, 2001; Hansasuta & Rowland-Jones, 2001; Simon *et al.*, 2006). Homosexual transmission is the main mode of transmission in Latin America and Southeast Asia, but is more rarely reported in Africa (Grant & De Cock, 2001). New infections are most likely to occur by transmission from someone who is also recently infected, with the estimates of such causality being suggested to be as much as 50% (Skar *et al.*, 2011). This elevated risk of transmission could be due to the exponential viraemia experienced in early infection, or the individual's lack of knowledge of their serostatus (Skar *et al.*, 2011). In developing countries there is also significant risk of transmission via blood donation, particularly in those countries where donation is remunerated. This transmission can be as a result of the receipt of infected blood products or during donation from insufficiently sterile equipment (Grant & De Cock, 2001; Hansasuta & Rowland-Jones, 2001). In industrialised countries the main mode of transmission remains through homosexual contact between MSM (Wolitski *et al.*, 2001; UNAIDS, 2010). Injection drug use is another important mode of transmission across many geographical regions, accounting for approximately one third of all HIV-1 infections outside SSA, being mostly localised to Eastern Europe and central and southeastern regions of Asia (Simon *et al.*, 2006; UNAIDS, 2010).

Transmission Dynamics

In heterosexual contact, transmission from man to woman is approximated to be eight times more likely than from woman to man (Hansasuta & Rowland-Jones, 2001). Vertical perinatal transmission is more prominent in developing countries than

industrialised regions mainly due to the quality of health care as it can be avoided through antiretroviral treatment (ART) and a good standard of obstetric management (Hansasuta & Rowland-Jones, 2001). Vertical transmission via breast-milk is highest in the early months of breastfeeding (Miotti *et al.*, 1999), which is a particular issue in developing countries where alternatives such as formula are expensive and difficult to obtain. This high vulnerability could stem from the child's immature immune system at this point. In homosexual contact, the majority of infections are observed in populations of MSM but have been observed in women who have sex with women; this is mainly thought to be due to be injection drug use and bisexual contact and there has been no evidence, thus far, of direct female-to-female transmission (Bevier *et al.*, 1995).

HIV PATHOGENESIS

There are four clinically definable stages to HIV infection; these are primary HIV infection (PHI), the chronic asymptomatic phase, symptomatic HIV infection, and AIDS (Hansasuta & Rowland-Jones, 2001; Harris & Bolus, 2008; Weber, 2001). These groups have been further broken down into diagnostically meaningful criteria by both the WHO (Harris & Bolus, 2008) and the United States Centres for Disease Control (CDC) (Mindel & Tenant-Flowers, 2001). Each stage is characterised by unique symptomology and viraemia; but may present differently in different geographical areas due to variations in opportunistic infections that are acquired during immunosuppression, and due to individual factors of the host (Grant & De Cock, 2001; Mindel & Tenant-Flowers, 2001).

Unless HIV is directly introduced to the blood stream (as in cases of needle-sharing, infected medical equipment, or *in utero* transmission), HIV is generally introduced to a new host through a mucosal barrier (Hansasuta & Rowland-Jones, 2001; Larsson, 2005; Lever, 2005; Weber, 2001). The cells that are most frequently and easily targeted by the virus are $CD4^+$ and $CD8^+$ T lymphocytes, macrophages and dendritic cells (Clapham & McKnight, 2001; Donaghy *et al*., 2006; Hel *et al*., 2006; Verani, *et al*., 2005). Thus, major players in cellular immunity and in antigenic presentation are the targets of HIV, two main aspects of immunity. At the mucosal barrier the virus is most likely to directly infect $CD4^+$ T-cells, or attach to dendritic cells (DC); most notably Langerhan's Cells (Derdeyn & Silvestri, 2005; Donaghy *et al*., 2006;

Hansasuta & Rowland- Jones, 2001; Larsson, 2005; Weber, 2001). If DC become infected, they then carry the virus to $CD4^+$ T-cells for eradication, but instead of eradicating the antigen, the $CD4^+$ T-cells then also become infected and then carry the virus to local lymph nodes where the virus replicates within other target immune cells (Donaghy *et al.*, 2006; Hansasuta & Rowland-Jones, 2001; Weber, 2001). HIV enters target cells by interacting with $CD4^+$ glycoprotein and a co-receptor of the seven transmembrane group, which are chemokine coreceptors; most notable of these are CCR5 and CXCR4 (Clapham & McKnight, 2001; Larsson, 2005; Lever, 2005; Reeves & Doms, 2002; Simon *et al*., 2006). Once in the cell, HIV replicates by using reverse-transcriptase to transcribe its viral ribonucleic acid (RNA) into proviral deoxyribonucleic acid (DNA). This proviral DNA is then inserted into the DNA of the host by a protein called *integrase* which is present in the HIV viral bundle along with *protease* (see figure two) (Lever, 2005; Harris & Bolus, 2008; Sierra *et al.*, 2005). After several weeks of this viral replication (between two and six weeks) a replication threshold is reached and viraemia proliferates through the body: inducing PHI (Hansasuta & Rowland-Jones, 2001; Weber, 2001).

Initially the viral population of HIV will be largely homogenous, although the virus is highly mutation-prone (Lawn, 2004). Once an HIV-specific T-cell response ($CD8^+$) has been initiated, any mutated virions that can evade cytotoxic T lymphocyte responses will proliferate (Lawn, 2004; Leslie *et al.*, 2004). $CD8^+$ T-cells are vital in the immune response to control the replication of HIV; however there is a constant battle between these cells and the rapid evolution of HIV virions (Derdeyn & Silvestri, 2005; Sierra *et al.*, 2005). Mutations also occur due to the "error-prone" viral protein *reverse-transcriptase* (RT), key to the retroviral reproduction of RNA to DNA (Lever, 2005; Roquebert *et al.*, 2009; Skar *et al.*, 2011). It is estimated that approximately 10 billion virions are produced each day in an infected individual, with each viral replication potentially diverging from the original *wild-type* to the point of one of 9600 nucleotides, or 0.1-0.3 mutations per genome and replication cycle (Lever, 2005; Roquebert *et al.*, 2009; Skar *et al.*, 2011). These mutations can be harmless, and may in fact reduce the viral fitness of the virion; but equally they could confer the virus to a state of increased fitness – and ultimately allow the virus to adapt extremely quickly to its environment (Lever, 2005; Roquebert *et al.*, 2009).

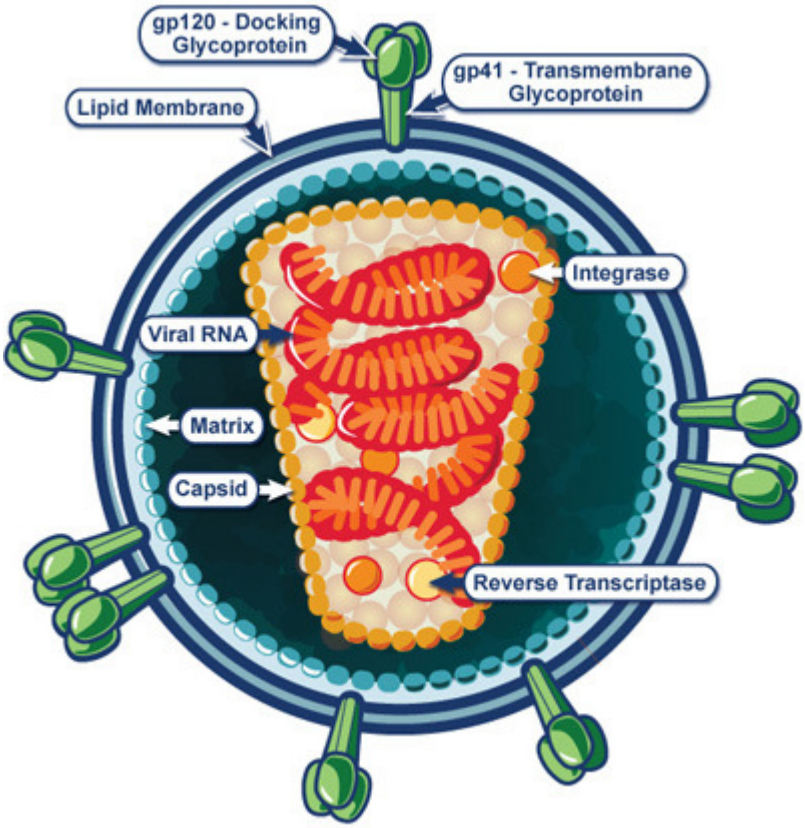

Figure 2. The internal structure of the HIV virion
Courtesy: National Institute of Allergy and Infectious Diseases

HIV is a lentivirus, like Epstein-Barr or Cytomegalovirus (CMV), but unlike these viruses, which live within the body without necessarily being fatal to the host, HIV causes catastrophic damage to the immune system resulting in death (Hel *et al.*, 2006; Levy, 2006). Healthy human immune systems can keep these other lentiviral infections in a latent, or dormant, state with the cellular immune response (mainly through $CD8^+$ T-cells); however HIV actively attacks and depletes this arm of immunity, making control of the infection impossible (Hel *et al.*, 2006; Levy, 2006). These other lentiviruses have been part of human virology for centuries, and it is thought that the reason HIV is deadly to humans is that it is relatively young, therefore neither humans nor the virus have had the chance to co-evolve in such away as to ensure mutual survival (Hel *et al.*, 2006; Levy, 2006).

Primary HIV Infection (PHI)

During this period HIV proliferates throughout the system by continuously invading $CD4^+$ immune cells and rapidly replicating (Harris & Bolus, 2008; Weber, 2001). This stage has presenting symptoms, which are usually characterised as fever, rash, swollen lymph nodes (lymphadenopathy), headaches, arthropathy, pharyngitis and weight loss (Hansasuta & Rowland-Jones, 2001; Harris & Bolus, 2008; Lewthwaite & Wilkins, 2005; Sierra *et al.*, 2005; Weber, 2001). These symptoms may be experienced in varying degrees of severity and will usually persist from a period of several days to several weeks (Hansasuta & Rowland-Jones, 2001; Harris & Bolus, 2008). Some patients will experience no symptoms at all, although the relative proportions of those who do not varies between studies (Lewthwaite & Wilkins, 2005; Sierra *et al.*, 2005; Weber, 2001). The symptoms that are presented in this phase are geographically specific, as are many of the symptoms presented in subsequent phases of HIV infection (Grant & DeCock, 2001; Hansasuta & Rowland-Jones, 2001).

The severity of symptoms during PHI has been positively associated with a higher rate of disease progression, and extreme cases may require temporary hospitalisation; particularly if the rate of $CD4^+$ T-cell destruction experienced in this phase causes immunosuppression leading to the acquisition of opportunistic infections (Hansasuta & Rowland-Jones, 2001; Harris & Bolus, 2008; Lewthwaite & Wilkins, 2005; Mindel & Tenant-Flowers, 2001). During this phase there is a massive activation and attrition of $CD4^+$ T-cells particularly in mucosal lymphoid tissues, and an extensive proliferation of virus within the plasma (Derdeyn & Silvestri, 2005; Hansasuta & Rowland-Jones, 2001; Hel *et al.*, 2006; Sierra *et al.*, 2005; Weber, 2001). There is also a large increase in activated circulating $CD8^+$ T-cells, with reports of virus-specific cytotoxic T-cells being evident as early as two days post-infection (Hansasuta & Rowland-Jones, 2001; Wilkinson & Gotch, 2001). The abnormal levels of $CD4^+$ and $CD8^+$ T-cells usually resolves after two to four weeks post-seroconversion whereby the levels return to an almost normal, but usually less than pre-infection, level (Hansasuta & Rowland-Jones, 2001; Harris & Bolus, 2008; Lewthwaite & Wilkins, 2005; Weber, 2001). Once an HIV-specific cellular immune response has been established by $CD8^+$ cytotoxic T-cells, the virus begins to come under control by the immune system (Simon *et al.*, 2006; Yamamoto & Matano, 2008). The plasma viraemia abates several weeks after seroconversion and then establishes a “set-point”

where it will remain more or less stable for a protracted period of time (Hansasuta & Rowland-Jones, 2001; Harris & Bolus, 2008; Sierra *et al.*, 2005; Weber, 2001). This stage may be particularly sensitive to cerebral influence, since the antibody-production has been repeatedly shown to be related to psychological factors (Cohen *et al*., 2001), a topic which will be fully discussed later.

The Chronic Asymptomatic Phase

This period of pathogenesis is variable from individual to individual, but can range from two to more than ten years after PHI (Derdeyn & Silvestri, 2005; Harris & Bolus, 2008; Lewthwaite & Wilkins, 2005; Mindel & Tenant-Flowers, 2001). During this phase of infection the individual continues to have a slow and steady rate of $CD4^+$ and $CD8^+$ T-cell decline with viral load (VL) remaining relatively stable, as viral replication still continues but at a slow rate due to high virion turnover (Lever, 2005; Sierra *et al.*, 2005; Weber, 2001).

The $CD4^+$ T-cell count during this phase is usually above $350/mm^3$ and the immune system is in a state of persistent activation thus providing the virus with a constant supply of infection targets (Derdeyn & Silvestri, 2005; Mindel & Tenant-Flowers, 2001). In some individuals, $CD4^+$ T-cell count has a rapid decline in the 12 months following PHI; this is thought to be caused by an R4 virus (i.e. a virus with CXCR4 tropism), rather than an R5 virus (CCR5 tropic virus) (Harris & Bolus, 2008; Lewthwaite & Wilkins, 2005). $CD8^+$ T-cells display a shorter half-life and higher turnover in HIV patients, particularly during this phase (Weber, 2001). Progression of the asymptomatic phase may be characterized by the individual feeling well but experiencing persistent generalised lymphadenopathy which can continue for several months, bilaterally across the body (Lewthwaite & Wilkins, 2005; Mindel & Tenant-Flowers, 2001). In the advance to symptomatic HIV infection the immunocompetency of the host begins to collapse, with the immune system growing increasingly unable to produce the cytokines (IL-2, interferon (IFN)) that are required to support T-cell generation (Harris & Bolus, 2008; Hel *et al.*, 2006; Weber, 2001).

Symptomatic HIV Infection

Before the status of HIV is replaced by that of AIDS, symptomatic HIV infection occurs. Here symptoms begin to appear and the host becomes susceptible to opportunistic pathogens (Mindel & Tenant-Flowers, 2001; Weber, 2001). The $CD4^+$ T-cell count of patients in the symptomatic phase will usually be between 200/mm^3 and 500/mm^3 (Harris & Bolus, 2008). Turnover of both $CD4^+$ and $CD8^+$ T-cells accelerates exponentially during this phase as the virus begins to replicate beyond the control of the immune system (Weber, 2001). Opportunistic infections take place due to the exhaustion of $CD4^+$ T-cell regeneration as a consequence of chronic T-cell activation, destroying T-cell homeostatic capabilities of the immune system (Hel *et al.*, 2006). The opportunistic infections that are experienced by the patient are geographically specific, with the access and effective use of highly active antiretroviral therapy (HAART) being another factor in this variance (Grant & De Cock, 2001; Mindel & Tenant-Flowers, 2001). The United States CDC has broken down this latter phase of HIV infection into clinically meaningful stages which are used for the diagnosis of AIDS with indicators of "AIDS defining illness". This is particularly useful in locations where sophisticated laboratory analyses may be difficult or impossible, so partial reliance on a clinical presentation can be an alternative (Grant & De Cock, 2001; Mindel & Tenant-Flowers, 2001).

Before AIDS defining illnesses occur, a number of opportunistic infections may present themselves. These are typically haematologic disorders, skin and mouth problems, and constitutional illnesses: usually diarrhoea, fever, weight loss and lethargy (Harris & Bolus, 2008; Lewthwaite & Wilkins, 2005; Mindel & Tenant-Flowers, 2001). The weight loss experienced during this stage is clinically distinct from HIV wasting syndrome, which is indicative of AIDS. AIDS-related HIV wasting syndrome is characterised by an at least 10% loss of body mass and either persistent fever or diarrhoea lasting for at least one month (Mindel & Tenant-Flowers, 2001).

AIDS

The stage of full-blown AIDS is characterised by a $CD4^+$ T-cell count of less than 200/mm^3 or the presence of at least one AIDS defining illness; any one of which could cause fatality in the immunocompromised patient (Harris & Bolus, 2008). AIDS defining illnesses that occur most commonly across all groups of HIV^+ patients

are; HIV wasting syndrome; HIV encephalopathy; tuberculosis; *pneumococcal pneumonia*; persistent oral, oesophageal and/or vaginal candidiasis; oral hairy leukoplakia (caused by active Epstein-Barr virus); *pneumocystis jirovecii* pneumonia; cerebral toxoplasmosis; Kaposi's sarcoma (herpes-derived neoplastic dermal lesions); severe diarrhoeal disease; *mycobacterium avium intracellulare*; systemic fungal infections; CMV infection; high-grade B-cell non-Hodgkin's lymphoma; and progressive multifocal leukoencephalopathy (cerebral viral infection caused by JC virus) (Davaro & Thirumalai, 2007; Grant & De Cock, 2001; Levine *et al.*, 2001; Lewthwaite & Wilkins, 2005; Mindel & Tenant-Flowers, 2001).

Coinfection

Coinfection in the HIV patient is very common, and varies according to geography. It is possible for an individual to become infected with more than one strain of HIV. Dual infections with HIV-1 and HIV-2, as well as with two variants of HIV-1 have already been well reported in the history of HIV, whereas dual infection with two forms of HIV-2 has not (Smith *et al.*, 2005; van der Kuyl & Cornelissen, 2007). Triple HIV-1 infections have also been observed in patients from Africa and Europe (van der Kuyl & Cornelissen, 2007), however these events are rare. HIV superinfection can also lead to viral recombination to create a new recombinant form, which can then be passed on to another individual (Blackard *et al.*, 2002; Smith *et al.*, 2005). Observations of coinfection with two distinct main groups of HIV (i.e. group M and group O) have been reported in several continents (Blackard *et al.*, 2002). Frequently in cases of dual HIV infection, the $CD4^+$ T-cell attrition is accelerated, along with viral load proliferation (Blackard *et al.*, 2002; Smith *et al.*, 2005; van der Kuyl & Cornelissen, 2007). Perhaps the most serious consequence of HIV dual- and super-infection is the scope for the virus to become drug-resistant or even multi-drug resistant (MDR) (van der Kuyl & Cornelissen, 2007). Two HIV virus strains can recombine to become a unique recombinant form (URF), which can be transmitted to others (van der Kuyl & Cornelissen, 2007). There have already been reports of patients with dual infection of MDR HIV strains (Smith *et al.*, 2005), along with the transmission of a MDR URF from a dual infected patient (dual infected with two MDR strains) to another individual (van der Kuyl & Cornelissen, 2007).

Additionally, accelerated pathogenicity of HIV has been observed with coinfections of bacterial (*mycobacterium tuberculosis:* TB), viral (hepatitis B and C viruses;

human T-cell lymphotropic virus), and parasitic (*plasmodium falciparum* malaria; *leishmania*; *schistosoma mansoni*; hookworm; lymphatic filariasis) origins; to name only a few (Brites *et al.*, 2009; Djoba Siawaya *et al.*, 2007; Harms & Feldmeier, 2002; Lawn, 2004; Nikolopoulos *et al.*, 2009; Petrovic, 2007; Rénia & Potter, 2006; Soriano *et al.*, 2010; Talaat *et al.*, 2008). These infections appear to create an additive effect, whereby the immune system is compromised further by HIV from mounting a response to these various infections – which can result in faster disease progression (Harms & Feldmeier, 2002). The immune system mounts a pro-inflammatory response (Th_2) to these infections, which not only provides target cells for HIV infection, but also inhibits anti-inflammatory (Th_1) responses required to control HIV viraemia (Djoba Siawaya *et al.*, 2007; Harms & Feldmeier, 2002; Lawn, 2004; Talaat *et al.*, 2008).

These infections pose a real threat to HIV^+ populations around the world. Hepatitis (both B and C variants) and Human T-cell Lymphotropic Virus are commonly found in HIV^+ patients, due to shared modes of transmission (Brites *et al.*, 2009; Nikolopoulos *et al.*, 2009; Soriano *et al.*, 2010). Recent estimates have placed the number of chronic Hepatitis C and HIV coinfections at 7 million (20% prevalence); with coincidence of chronic Hepatitis B and HIV estimated at 3 million, ranging between 5% prevalence in Western regions up to 20% in developing regions such as SSA and South East Asia (Soriano *et al.*, 2010). TB is both an opportunistic infection associated with HIV infection (a leading cause of HIV mortality), and a coinfection observed frequently in HIV^+ patient populations (Djoba Siawaya *et al.*, 2007; Lawn, 2004). Parasitic infections are common in tropical and desert areas, making the interactions of these infections particularly pertinent to the millions of HIV^+ people living in these areas (Harms & Feldmeier, 2002; Lawn, 2004; Talaat *et al.*, 2008). It is also important to note that the areas where these parasitic infections are commonly found are also usually areas of lower income, with less access to healthcare and medications – yet another addition to the relative disadvantages experienced by HIV^+ patients in these locales compared to those from industrialised nations.

FACTORS RELATING TO SUSCEPTIBILITY & RESISTANCE

Susceptibility

Mucosal Immune Status & Genetics

It is supposed that most sexual exposures to HIV do not result in infection (Kaul *et al.*, 2008) and therefore there must be specific factors involved in the risk of transmission of the virus from one body to another. There are two perspectives to increased susceptibility; that of increased likelihood of the donor transmitting the virus, and that of increased likelihood of the individual receiving the virus (Hansasuta & Rowland-Jones, 2001; Kaul *et al.*, 2008).

Transmission Probability

Increased infectivity of an HIV donor is most influenced by the plasma HIV RNA VL of the infected individual. Higher VL indicates increased likelihood of transmission, which is thought to be due to a high correlation between plasma and genital secretion VLs (Hansasuta & Rowland-Jones, 2001; Kaul *et el.*, 2008). It is also thought that co-infections with other sexually transmitted infections (STIs) and vitamin A deficiency increases the VL of genital secretions (Hansasuta & Rowland-Jones, 2001; Kaul *et al.*, 2008). For example, genital ulceration caused by STIs (i.e. gonorrhoea, syphilis and genital herpes (HSV2)) is likely to increase the amount of viral shedding in genital secretions (Hansasuta & Rowland-Jones, 2001; Kaul *et al.*, 2008; Lawn, 2004). CMV is another STI that is associated with increased VL in genital secretions, an infection that is often found in those with HIV (Kaul *et al.*, 2008).

Contraction Susceptibility

In terms of enhanced susceptibility to HIV infection, the main factors involved are related to mucosal immune status and genetic factors (Derdeyn & Silvestri, 2005; Hansasuta & Rowland-Jones, 2001; Kaul *et al.*, 2008; Lama & Planelles, 2007; Spear *et al.*, 2007). Increased susceptibility related to mucosal immune status is directly associated with co-infections of STIs such as CMV, syphilis, HSV2, gonorrhoea and bacterial vaginosis (Hansasuta & Rowland-Jones, 2001; Kaul *et al.*, 2008; Spear *et al.*, 2007). This is largely supposed to be due to an increase in HIV target immune

cells in the genital mucosae, as the immune system attempts to control these local infections (Hansasuta & Rowland-Jones, 2001; Kaul *et al.*, 2008; Spear *et al.*, 2007). Hormonal changes also affect mucosal immune status in women. During the follicular phase of the menstrual cycle, when ovarian oestrogen levels increase, higher numbers of immature DCs can be found on the cervix making women more vulnerable to infection during this time (Kaul *et al.*, 2008). Additionally, during the secretory phase when progesterone is abundant (but DC levels have diminished), CCR5 is expressed in higher numbers across the cervical tissues, also increasing transmission susceptibility in viruses that use this co-receptor (Kaul *et al.*, 2008). This increase in susceptibility related to increased levels of progesterone is also thought to explain the observed associations between increased HIV infection susceptibility and hormonal contraception, which is usually through the introduction of progesterone (Hansasuta & Rowland-Jones, 2001; Kaul *et al.*, 2008).

Genetic Factors

Genetic factors that impact on the susceptibility and resistance to HIV infection are still being discovered. Thus far the majority of genetic studies have uncovered factors relating to resistance, but there have been some key discoveries relating to susceptibility (Derdeyn & Silvestri, 2005; Hansasuta & Rowland-Jones, 2001). Research in Africa has uncovered that enhanced transmission and susceptibility have been associated with the sharing of the class I HLA-B (Human Leukocyte Antigen B) allele between partners (Derdeyn & Silvestri, 2005; Dorak *et al.*, 2004; Hansasuta & Rowland-Jones, 2001). These probably depend on the resemblance between the virus's and the host's HLA. Conversely, there is also a protective factor associated with the HLA alleles insomuch as the foreign HLA molecules on the virus may trigger a rejection reaction (similar to that observed in failed organ transplant), allowing resistance, or may make infection more efficient if the virus evades the initial cytotoxic T-lymphocyte ($CD8^+$) response (Derdeyn & Silvestri, 2005; Hansasuta & Rowland-Jones, 2001).

In a similar dichotomous fashion, polymorphisms in the promoter region of RANTES (a gene coding for an HIV suppressing chemokine) have been found to influence susceptibility and resistance (Hansasuta & Rowland-Jones, 2001). It is suggested that the polymorphism leads to increased expression of RANTES which can lead to

increased inflammation of mucosae, thus leading to increased susceptibility, but can also lead to higher suppression of the virus if infection takes place (Hansasuta & Rowland-Jones, 2001; Lama & Planelles, 2007). Thus, the RANTES polymorphysim can affect risk of infection, depending on the level of elicited immune responses and its timing in relation to HIV-infection.

Variations in the CCL3L1 gene may also account for decreased susceptibility, as this gene encodes for MIPα (macrophage inflammatory protein α), and greater amounts of this chemokine may exhibit competitive usage of the CCR5 co-receptor, weakening HIV's bonding to $CD4^+$ immune cells (Simon *et al.*, 2006). Another finding concerns a polymorphism in the promoter region of CCR5, which, if apparent, is associated with increased susceptibility of children contracting HIV from their mothers during gestation (Hansasuta & Rowland-Jones, 2001).

Resistance

Genetics

The study of resistance factors relating to HIV infection has been greatly aided by the research of two groups of individuals; long-term non-progressors (LTNPs) and highly-exposed persistently-seronegative (HEPS) individuals (Hansasuta & Rowland-Jones, 2001; Lama & Planelles, 2007). LTNPs represent approximately two to four percent of the HIV infected population. They are infected with HIV but live for a protracted period of time (over ten years) without any symptoms even in the absence of treatment and typically have $CD4^+$ T-cell counts of over $500/mm^3$ with plasma VL below 10,000 RNA copies per millilitre (Lama & Planelles, 2007; Wilkinson & Gotch, 2001). HEPS are individuals who fail to contract HIV despite either numerous or persistent exposures to the disease. They have been found in populations of HIV^- children of HIV^+ mothers, injection drug users, sex workers, those having high levels of unprotected sex, and health workers who have undergone accidental exposure to the virus (Lama & Planelles, 2007).

The most significant finding in the genetic studies has been the identification of polymorphisms of the 32 base pair of the CCR5 gene (i.e. CCR5Δ32) which enables protection from HIV in homozygotes (i.e. those with the full pair deleted) and protection from HIV progression in heterozygotes (i.e. those with one of the pair deleted) (Clapham & McKnight, 2001; Donaghy *et al.*, 2006; Hansasuta & Rowland-

Jones, 2001; Lama & Planelles, 2007; Sierra *et al.*, 2005; Simon *et al.*, 2006). Those who are CCR5Δ32^{+} have been known to acquire HIV, but it is thought that these viruses are CXCR4-tropic (R4) strains, which are more rare than the CCR5-tropic (R5) strains (Clapham & McKnight, 2001; Hansasuta & Rowland-Jones, 2001; Lama & Planelles, 2007). It has been observed that HEPS are over-represented as a group in those who are CCR5Δ32 homozygous, and LTNPs are similarly so in those who are CCR5Δ32 heterozygous, but this is not universal, so there may be other factors involved that are as yet undiscovered (Lama & Planelles, 2007). CCR5Δ32 polymorphisms are found most commonly in Caucasian groups, particularly those from northern Europe, and are almost never found in African or Asian groups, further explaining the high prevalence of HIV and AIDS in the latter regions (Hansasuta & Rowland-Jones, 2001; Lama & Planelles, 2007). LTNPs have also been found to possess a polymorphism of the CCR2 allele V63I phenotype, which appears to slow the progression to AIDS (Sierra *et al.*, 2005). HLA molecules are also implicated in the phenomenon of long-term non-progression. LTNPs have been found to have increased amounts of HLA classes I and II molecules (Sierra *et al.*, 2005). This suggests that antigen-presentation, the main role of HLA, is important in protecting against the progression of HIV, either by more frequent/efficient antigen-presentation or more frequent/efficient activation of healthy CD4^{+} T-cells.

TREATMENT OF HIV INFECTION

HIV Surveillance & Pharmacotherapy

Medical care of HIV patients in developed countries is mostly comparable, with patients being seen every three to six months, and their CD4^{+} T-cell count, VL and weight being checked at each visit (Mindel & Tenant-Flowers, 2001). This may not be possible in more economically-strained regions, where the recommendation is for the patient to attend medical monitoring as often as is possible for that region's resources (Grant & De Cock, 2001). The selection of the type of treatment, and when it should begin is often highly individual and can depend on the particular case and the health resources of the geographical setting (Grant & De Cock, 2001; Harris & Bolus, 2008; Simon *et al.*, 2006). Hammer *et al.* have published guidelines as part of the International AIDS Society, stating that treatment should be started in all

symptomatic patients, and in those asymptomatic patients who have $CD4^+$ T-cell counts of between 350/mm^3 and 200/mm^3 (Hammer *et al.*, 2006; Harris & Bolus, 2007; Simon *et al.*, 2006).

Treatment for HIV first started with simple Antiretroviral Treatment (ART), but due to the ability of the virus to form drug-resistant recombinants and to replicate at a high rate (Paredes & Clotet, 2010), a race often ensues between the development of safe and effective drugs and the evolution of the virus to resist them. Developments in the last decade that have seen combinations of antiretrovirals (ARVs) being used to great benefit, and these combinations are now referred to as Highly Active Antiretroviral Treatment (HAART) (Harris & Bolus, 2008; Simon *et al.*, 2006; Weller & Williams, 2001). To date there are so far six different drug classes used in HAART; nucleoside reverse-transcriptase inhibitors (NRTIs), nucleotide reverse-transcriptase inhibitors (NtRTIs), non-nucleoside reverse-transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors (FIs) and co-receptor inhibitors (CRI) (see table one) (De Clercq, 2010; Deeks, 2006; Orsega, 2007; Wilkinson & Gotch, 2001). Today these drugs now also come in fixed-dose combinations, allowing a vast reduction in pill-burden, and easy combination with other drug classes (Cihlar & Ray, 2010; Ganguli *et al.*, 2011). The goal of antiretroviral treatment is to control viral replication, which in turn helps to repopulate $CD4^+$ T lymphocyte levels (Deeks, 2006; Simon *et al.*, 2006) The efficacy of these treatments is evaluated through the monitoring of both T-cell levels and VL assay. See table one for a summary of the currently approved HAART medications available within the European Union.

Nucleoside Reverse-Transcriptase Inhibitors (NRTIs)

NRTIs and, more recently, NtRTIs (described below) form the staple of modern combination ARV therapy (Cihlar & Ray, 2010). NRTIs prevent viral replication by both inhibiting the protein RT, which is required to translate HIV RNA into DNA, and terminating the DNA chain by interrupting its synthesis (Deeks, 2006; Harris & Bolus, 2008; Weller & Williams, 2001). However HIV has adapted to this, the oldest form of antiretroviral therapy, and new strains have emerged which are less vulnerable to RT inhibition (Deeks, 2006; Weller & Williams, 2001). The use of these drugs is also limited due to polydrug interactions and the numerous and diverse adverse side-effects experienced by patients (Cihlar & Ray, 2010).

Nucleotide Reverse-Transcriptase Inhibitors (NtRTIs)

Tenofovir, marketed as *Viread*, is the only approved drug in this class to date, has been formulated as a component of two fixed-dose combination drugs (*Truvada* and *Atripla*) and is now one of the most frequently prescribed drugs in HAART today (De Clercq, 2009; Pozniak, 2008). The compound has the same mechanism of action as NRTIs, but it is based on nucleotide analogues as opposed to nucleoside, making it effective in activated and resting cells (De Clercq, 2009; Pozniac, 2008). The drug is comparatively well tolerated, and as it has a relatively long half-life there are fewer implications if the patient does not take the drug exactly on time (Pozniac, 2008). As well as being approved for use in HIV-1 and HIV-2, it has also been approved for use in Hepatitis B infection (De Clercq, 2009; Pozniac, 2008) making this drug exceptionally useful for those coinfected with both viruses.

Non-Nucleoside Reverse-Transcriptase Inhibitors (NNRTIs)

NNRTIs work in a similar fashion, targeting RT, but these are distinct in their ability to bind to the catalytic non-active site of the enzyme, disrupting the enzyme activity from creating conformational changes (Harris & Bolus, 2008; Weller & Williams, 2001). These agents are highly effective at disrupting viral replication and have relatively low toxicity to the patient, but they are only effective at this action with HIV-1 and not HIV-2 (Weller & Williams, 2001). NNRTIs can be used well with other classes of HAART, but again suffer in efficacy from HIV mutations which are resistant to their action and so often require combination with other chemical agents (Weller & Williams, 2001).

Table 1. A summary of currently available drugs within the HAART repertoire of the European Union. Drug class, name(s) and mechanism of action (HIV target) are provided.

Drug Class	Generic Name(s)	Trade Name(s)	Target
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	Zidovudine (AZT) Lamivudine (3TC) Abacavir Stavudine (d4T) Didanosine (ddl) Emtracitibine (FTC)	*Retrovir* *Epivir* *Ziagen* *Zerit* *VidexEC* *Emtriva*	Viral *Reverse Transcriptase*
Nucelotide Reverse Transcriptase Inhibitor (NtRTI)	Tenofovir	*Viread*	Viral *Reverse Transcriptase*
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)	Efavirenz Etravirine Nevirapine	*Sustiva/Stocrin* *Intelence* *Viramune*	Viral *Reverse Transcriptase*
NRTI/NtRTI Fixed-Dose combinations	FTC/Tenofovir 3TC/AZT 3TC/Abacavir 3TC/Abacavir/AZT	*Truvada* *Combivir* *Kivexa* *Trizivir*	Viral *Reverse Transcriptase*
NRTI/NtRTI/NNRTI Fixed-Dose combination	FTC/Tenofovir/Efavirenz	*Atripla*	Viral *Reverse Transcriptase*
Protease Inhibitors (PIs)	Atazanavir Darunavir Fosamprenavir Indinavir Lopinavir/Ritonavir Ritonavir Saquinavir Tipranavir	*Reyataz* *Prezista* *Telzir* *Crixivan* *Kaletra* *Norvir* *Invirase* *Aptivus*	Viral *Protease*
Fusion Inhibitor (FI)	Enfuviritide (T-20)	*Fuzeon*	Viral integration in to host cell DNA
CCR5 Inhibitor	Maraviroc	*Celsentri*	Disruption of the interaction between CCR5 and HIV protein gp120 ("docking" protein)
Integrase Inhibitor	Raltegravir	*Isentress*	Viral *Integrase* protein

Protease Inhibitors (PIs)

PIs target the protease enzyme in the viral bundle of HIV that is used to let the virus "bud" out of an infected cell once it has successfully invaded and replicated in the target cell cytoplasm (Harris & Bolus, 2008; Lever, 2005; Weller & Williams, 2001).

As protease is inhibited, it cannot break down the strands of viral proteins that mature the virions (a process known as protein catabolism), and so only immature, inert viral proteins are released (Harris & Bolus, 2008; Simon, Ho & Karim, 2006; Weller & Williams, 2001). PIs are very efficient in preventing viral proliferation, and their introduction to mainstream medicine was associated with significant decreases in both morbidity and mortality in HIV patients (Weller & Williams, 2001). As with the RT inhibitors, HIV has evolved and developed some degree of resistance to PIs, and that in combination with the tolerability of the drug class have created problems in recent years (Weller & Williams, 2001).

Fusion Inhibitors (FIs)

FIs are one of the newest classes of HAART drugs and work by preventing the fusion and entry to target cell membranes (Harris & Bolus, 2008; Orsega, 2007; Rockstroh & Mauss, 2004; Simon, Ho & Karim, 2006). The purpose of FIs is to effectively block HIV binding to chemokine coreceptors (CD4, CCR5, CXCR4), and also disrupt the fusion mechanics of the HIV virion, preventing bonding between the two cell membranes (Harris & Bolus, 2008; Rockstroh & Mauss, 2004). To date there is only one approved drug in this class, enfuvirtide (*fuzeon*), its administration requires twice-daily injections and is usually reserved for those patients who have developed resistance to other drug classes as it can help to overcome this resistance (Deeks, 2006; Orsega, 2007; Rockstroh & Mauss, 2004; Simon, Ho & Karim, 2006).

Integrase Inhibitors (INIs)

So far there has only been one INI developed beyond stage III clinical trials and approved for use in humans; raltegravir (*Isentress*) (De Clercq, 2010; Hicks & Gulick, 2009; Ramkumar *et al.*, 2010). This class of drug is designed to target the integration of the proviral DNA into the host genome, but is only approved for use in HIV-1 (De Clercq, 2010; Hicks & Gulick, 2009). It is currently only recommended for use in patients who are treatment-experienced, with particular emphasis towards those who have contracted or developed MDR virus (De Clercq, 2010; Ramkumar *et al.*, 2010). Raltegravir has been relatively well tolerated by patients, and does not involve polydrug interactions like so many other ARV drugs (Hicks & Gulick, 2009;

Ramkumar *et al.*, 2010). Unfortunately resistance to raltegravir has been observed, in relatively low opposition (due to just a few mutations in HIV *integrase*) (De Clercq, 2010).

Co-Receptor Inhibitors (CRIs)

Maraviroc (*Cesentri*) is the first CRI available worldwide, working in the same fashion as INIs, but specifically targeting the chemokine coreceptor CCR5 (Gilliam *et al.*, 2010; Ray, 2008). Due to its unique chemical target, this drug is only effective for R5 HIV strains, and offers no effect for those strains that target CXCR4 or both chemokine coreceptors, however R4 and R4R5 viruses are rare by comparison (Ray, 2008). Maraviroc essentially works as a receptor antagonist; thereby blocking the receptor (Gilliam *et al.*, 2010; Ray, 2008). Currently Maraviroc is reserved for treatment-experienced patients, used largely as a "salvage" regimen, but is tolerated well and can be used in combination with other drugs for the treatment of viruses other than HIV (Gilliam *et al.*, 2010; Ray, 2008). The resistance to this drug is an issue currently being researched, with suggestions that its use may trigger a *de novo* receptor-tropism switch (i.e. R5 to R4), however this switch does occur naturally in more advanced cases of HIV and use of Maraviroc does not so far appear to have accelerated these findings (Ray, 2008).

Efficacy Issues

As effective as these drugs are at combating the progression of HIV disease, they also present problems in terms of tolerance and toxicity, which can lead to difficulties in patient adherence – an aspect which is vital for clinical efficacy (Deeks, 2006; Lewthwaite & Wilkins, 2005; Simon *et al.*, 2006; Weller & Williams, 2001). Resistance of the virus to every drug has been observed so far, once it has become tolerant to its effects; but there have also been some completely drug-resistant strains of the virus which have been found in up to 20% of new infections in countries with good access to antiretroviral agents (Simon *et al.*, 2006). Each class of drugs has associated toxicities, but some are also drug-specific (Deeks, 2006; Weller & Williams, 2001). Immune reconstitution inflammatory syndrome is being increasingly seen amongst those who are in the early stages of HAART and is still not properly

understood, but it is thought to be a reaction from a sudden increase in immune cells (Davaro & Thirumalai, 2007; Deeks, 2006).

Among NRTIs the class-associated toxicities are lactic acidosis, lypodystrophy and hepatic steatosis (fatty liver disease) (Davaro & Thirumalai, 2007; Deeks, 2006; Simon *et al.*, 2006; Weller & Williams, 2001). The more severe of the drug-related toxicities in this class are peripheral neuropathy (*stavudine*; *zalcitibine*; *didanosine*), nausea (*zidovudine*; *abacavir*), hepatitis (*stavudine*), pancreatitis (*didanosine*) and myopathy (*zidovudine*) (Deeks, 2006; Weller & Williams, 2001). NNRTIs have class-associated toxicities similar to NRTIs, the more severe drug-specific toxicities are hepatitis (*nevirapine*), mood disturbances (*efavirenz*) and Stevens-Johnson syndrome (a necrotising skin condition) (*nevirapine*) (Deeks, 2006; Weller & Williams, 2001). PIs are associated with hyperlipidaemia (high blood lipid content), lypodystrophy and diabetes mellitus as a class (Deeks, 2006; Weller & Williams, 2001). The more severe drug-associated toxicities of this group include chronic diarrhoea (*nelfinavir*; *ritanovir*; *amprenavir*; *lopinavir*), hepatitis (*ritanovir*), and nephrolithiasis (kidney or bladder stones) (*indinavir*) (Deeks, 2006; Weller & Williams, 2001). FIs are generally well accepted by the patient, but they do have adverse effects in terms of their parenteral administration, which can cause severe reactions at injection sites (Deeks, 2006; Rockstroh & Mauss, 2004).

Therapeutic Recommendations

The therapeutic strategy that is recommended for these drugs is the use of at least one drug from at least two of the agent classes but these guidelines can differ according to the individual, particularly in cases of pregnancy, infection in children and co-infection with hepatitis (Deeks, 2006; Lewthwaite & Wilkins, 2005; Sierra *et al.*, 2005). In order to combat the difficulties experienced with drug toxicity and resistance, structured treatment interruptions can be implemented (Deeks, 2006; Simon *et al.*, 2006; Wilkinson & Gotch, 2001). This is generally only recommended in those patients who have well-controlled viraemia or who would benefit physically more from stopping than from continuing with treatment (Deeks, 2006; Simon *et al.*, 2006; Wilkinson & Gotch, 2001). This treatment strategy is still being investigated as it is thought that interruptions may encourage the re-constitution of viral reservoirs within the body that will ultimately make the condition more difficult to treat (Wilkinson & Gotch, 2001).

FUTURE DIRECTIONS IN HIV RESEARCH

Vaccine Development

As with all communicable diseases, one of the ultimate goals for HIV medicine is a vaccine. There have been many vaccine trials to date in both HIV and SIV, but none have yielded sufficiently positive efficacy to be brought to market (Virgin & Walker, 2010). Most importantly, the correct immunological pattern has not yet been discovered which will allow the blanket recognition and defense against HIV (Burgers *et al.*, 2011; Virgin & Walker, 2010). It has also not been decided whether the most feasible or appropriate end-goal would be immunity against contracting HIV, or prevention of disease development to AIDS (Virgin & Walker, 2010).

General vaccine strategy involves the programming of memory T and B cells to recognise an antigen, however this approach is redundant in the case of HIV due to the aforementioned "error-prone" reverse-transcriptase (RT) enzyme of HIV (Virgin & Walker, 2010). The HIV protein RT is relatively unstable, and as the virus is transcribed and reproduced, the encoding of this enzyme is prone to mutation, making each virion potentially different (Lever, 2005; Roquebert *et al.*, 2009; Skar *et al.*, 2011). Another hurdle to surmount in vaccine research comes from the latent reservoirs of infected cells, characteristic of lentiviruses, which allow the evasion of infected cells from immune surveillance (Virgin & Walker, 2010). Nonetheless, vaccine developments and trials continue to be conducted in the hopes that a successful vaccine can be developed, and to date one trial has provided cautious optimism in the field. A vaccine study in Thailand in a large sample of volunteers showed a modest level (31%) of protection against HIV-1 infection as compared to a placebo group (Rerks-Ngam *et al.*, 2009).

Pre-Exposure Prophylaxis (PrEP)

One of the most recent avenues of interest in HIV medicine is that of pre-exposure prophylaxis (PrEP), or the ability to prevent infection before exposure occurs (Naswa & Marfatia, 2011). Two trials of PrEP have been conducted so far, with encouraging results. The first study employed a tenofovir (NtRTI) based gel, to be applied topically before sexual contact. This study used an experimental group (N=445) versus control placebo group (N=444) of women in South Africa (Karim *et al.*, 2010). They found an overall reduction in HIV infection of 39%, ranging between 28-54% depending on adherence (Karim *et al*, 2010). The second study was conducted using

2499 MSM and transgender women who have sex with men in samples on four continents (South and North America, South East Asia and Africa) (Grant *et al.*, 2010). The study employed a PrEP strategy in HIV seronegative participants, versus a placebo, using Emtracitibine (a NRTI) and tenofovir (NtRTI) in pill form, and found that this regimen provided a 44% reduction in infection rates (Grant *et al.*, 2010).

SUMMARY

The management of HIV has been part of mainstream medicine for 30 years now, and although the scientific community has developed a wealth of knowledge about the disease, there is still much to be uncovered. It is a very complex virus, creating an even more complex somatic experience, highly variable across individuals. By effectively hijacking the immune system, using its innate mechanisms for antigen control, it has proved to be a formidable foe in medicine. The fact that it proliferates and thrives using the same system used and developed to control viral spread; and the fact that each new virion can be potentially different to the parent strain, thus evading antibody strategy, provide challenging conditions in pharmacy and vaccine development. Additional complexities are apparent when considering the impact that host genetics and immunity have on the tremendous variability of the pathogenicity of the virus.

Despite the exponential advances made in the treatment of the infection, a cure still remains elusive. It is, however, a remarkable feat of contemporary research and scientific understanding that such a relatively young disease has been met with the development of over 25 different approved pharmacotherapies, prolonging wellness and life far beyond that which was the norm at the beginning of the epidemic. In the absence of remedy or vaccine it is necessary that interventions, both medical and behavioural, be developed and implemented on a wide scale that target prevention of infection and effective management of illness. Pharmacotherapy is being continually developed and different approaches to a vaccine are still being considered and tested, but in the absence of a unilateral cure, efforts must be continued to ensure that those living with the disease have adequate access to treatment and can continue to live with a maximum quality of life.

Chapter 2

Host Psychosocial Predictors of Prognosis in HIV Infection

Human Immunodeficiency Virus (HIV) infection is a chronic, progressive illness, which results in the development of Acquired Immune Deficiency Syndrome (AIDS), and subsequently death (Graham, 1998; Harris & Bolus, 2008; Levy, 2006). Whilst the progression from HIV infection to a clinical AIDS status is characterised by a distinct and discrete categorisation of disease stages, the rate at which the illness progresses is highly variable and is still not fully understood (Ashton *et al.*, 2005; Farinpour *et al.*, 2003; Lama & Planelles, 2007; Langford *et al.*, 2007; Leserman, 2003). Those factors that are thus far understood to determine the trajectory of the disease progression are: viral factors (Derdeyn & Silvestri, 2005; Simon *et al.*, 2006); host genetic and biological factors (Derdeyn & Silvestri, 2005; Donaghy *et al.*, 2006), and host psychosocial factors, which can modulate pathogenesis through psychoneuroimmunological mechanisms (Langford *et al.*, 2007; Leserman, 2003; Soloman *et al.*, 2000).

The plethora of host psychosocial HIV disease progression modulators have received broad attention in recent years, particularly since the advent of Highly Active Antiretrovial Treatment (HAART) which has led to much more stable medical management of HIV pathogenesis and morbidity (Hartzell *et al.*, 2008; Ironson *et al.*, 2005b). Since HAART has become widely available in the developed world, the life expectancy of those with HIV has become greatly increased, although there still exists a large heterogeneity in this life expectancy (The Antiretroviral Therapy Cohort Collaboration, 2008; Balbin *et al.*, 1999; Farinpour *et al.*, 2003). This has led to the increased focus on host psychosocial factors on both morbidity and mortality in HIV and the predictors of HAART adherence, a factor that is vital to its efficacy and therefore survival in HIV (Harris & Bolus, 2008; Simon *et al.*, 2006). Demographic differences have been related to variations in prognosis, with factors such as younger age, higher IQ and being female associated with slower disease progression (Farinpour *et al.*, 2003; Langford *et al.*, 2007). These demographic factors are useful in our understanding of the variance of the disease course, but cannot be changed and so in terms of identifying variables susceptible to invertention in HIV, psychosocial predictors provide valid basis for research and intervention.

Broadly speaking, the main components of host psychosocial influences on HIV progression fall in to three categories; psychological (e.g. depression, anxiety, optimism), social (e.g. social support, social identity), and behavioural (e.g.

abuse/dependence of drugs and/or alcohol, HAART adherence, health behaviour). These components influence each other to some degree, for example lack of social support may induce depression, which can in turn impact on adherence; but can also exert their effects independently.

PSYCHOLOGICAL FACTORS

Psychological Morbidity

The diagnosis of a life-limiting illness such as HIV is highly associated with the development of psychological morbidities, such as depression, anxiety and sometimes Post Traumatic Stress Disorder (PTSD) (Gibbie *et al.*, 2006; McCain *et al.*, 2003; Milam, 2006). Even if an individual manages to receive such a diagnosis without developing a psychological condition, the process of living with HIV and its associated physical wellness, lifestyle and social changes leave the individual more vulnerable to experiencing psychological morbidity (Chippindale & French, 2001). It is also suggested that those people who become infected with HIV are more likely to come from backgrounds associated with psychological morbidity, such as a history of sexual and/or physical abuse and drug or alcohol dependence, which is reported significantly more in HIV-seropositive (HIV^+) individuals than in the general population (Cohen *et al.*, 2000; Dévieux *et al.*, 2007; Kimerling, *et al.*, 1999; Mugavero *et al.*, 2007; Pence, 2009). As the mechanisms by which these psychological morbidity factors impact on HIV disease are often shared amongst these factors, they will be discussed after the summary.

Depression

It is estimated that up to 50% of the HIV^+ population are also living with some sort of depressive disorder (Evans *et al.*, 2002; Gibbie *et al.*, 2006; Hartzell *et al.*, 2008; Pence, 2009). Depression in HIV^+ patients has been reported to be five to ten times more prevalent than in the general population (Pence, 2009). This prevalence rate constitutes a significant health problem, particularly in view of the adverse effects of some HAART medications, which can elicit depression in otherwise mentally healthy patients (e.g. Efavirenz, Zidovudine) (Hartzell *et al.*, 2008; Kopnisky *et al.*, 2004; Montessori *et al.*, 2004). Furthermore, it is estimated that nearly 50% of HIV^+ patients

who meet diagnostic criteria for a depressive disorder are undiagnosed (Asch *et al.*, 2002; Pence, 2009). This further highlights health care problems in these patient groups if depression is a contributor to disease course, or, indeed, a barrier to optimum treatment efficacy.

The current consensus is that depression in HIV^+ patients has a negative effect on mortality, neurocognitive functioning, HIV-related immune response and disease severity (subjective experience), and faster progression toward an AIDS diagnosis or diagnosis of an AIDS-defining condition, which is proportionately more pronounced in those with more chronic and severe depression than those with more mild or intermittent depression (Greeson *et al.*, 2008; Hartzell *et al.*, 2008; Ironson, *et al.*, 2005b; Leserman, 2003; Pence, 2009). There has been some evidence to suggest that HIV pathogenesis may influence mood via inflammation. Inflammation is a key aspect of HIV disease and contributes largely to non-opportunistic morbidity and mortality (i.e. morbidity and mortality unrelated to opportunistic infections or neoplasia) (Lane, 2010). Further, systemic inflammation has been demonstrated to cause negative affectivity (both anxiety and depression); with these effects being suggested to be driven by the increase in proinflammatory cytokines such as tumour necrosis factor (TNF) (Reichenberg *et al.*, 2001). Therefore, the characteristic inflammation experienced in HIV may contribute to both psychological and physiological morbidity.

Anxiety & Stress

Anxiety and stress have been known to affect immune functioning across a variety of illness states for many years (Balbin *et al.*, 1999; Hassan & Douglas, 1990; Leserman, 2003; Stein & Rotheram-Borus, 2004; Vassend & Eskild, 1998; Vassend *et al.,* 1997). HIV is an illness that is highly associated with stressors, such as social stigma, victimisation, excessive medication burdens, changes in personal relationships and shortened life expectancy (Hassan & Douglas, 1990; Kimerling *et al.*, 1999; Stein & Rotheram-Borus, 2004). This means being able to understand the effect that anxiety exerts on HIV disease course is essential.

The link between excessive stress and HIV disease progression is by far the most supported in the spectrum of psychosocial disease prognostics in this field (Balbin *et al.*, 1999). Research has suggested that those experiencing higher levels of life stress

can progress through HIV disease at up to four times the rate of those with intermediate or low levels of life stress will (Balbin *et al.*, 1999; Leserman, 2003). It has been observed that several immune parameters are related to increased experience of life stressors in HIV disease; $CD4^+$ (helper) T-lymphocyte decline, $CD8^+$ (cytotoxic) T-lymphocyte population and functional decline, and natural killer (NK) cell decreased number, activity and function (Balbin *et al.*, 1999; Greeson *et al.*, 2008; Soloman *et al.*, 2000).

As anxiety and stress are associated with rises in cortisol secretion and proliferation; there is also a direct correlation between the levels of stress-associated plasma cortisol and prognostic indicators of HIV disease (progression to AIDS, to clinical progression, and to death) (Leserman *et al.*, 2002). One common theme amongst the literature is bereavement-associated anxiety; which has been shown to have very strong links to $CD4^+$ T-cell decline and HIV disease progression (Balbin *et al.*, 1999; Kopnisky *et al.*, 2004; Leserman, 2003). Another specific type of stressor has been identified in a study involving HIV^+ women. Here "victim status" was identified as being strongly related to subjective health status, with those women who had reported at least three types of victimisation (e.g., burglary, rape, mugging) being shown to experience higher rates of AIDS defining conditions (by self-report and medical evaluation) in comparison to HIV^+ women who had not reported victimisation (Kimerling *et al.*, 1999).

Post-Traumatic Stress Disorder (PTSD)

PTSD has been observed as a reaction to HIV diagnosis up to eight years post-diagnosis (Delahanty *et al.*, 2004; Kelly *et al.*, 1998; Milam, 2006; Safren *et al.*, 2003) and has been reported in HIV^+ populations irrespective of its origins (Kimerling *et al.*, 1999; Pence, 2009). Because of the difficulties in forming a diagnosis of PTSD that is clinically distinct from sustained anxiety, there is a paucity of studies concerning the disease modulating effects of PTSD (Kelly *et al.,* 1998; Pence, 2009). With this in mind, the findings of high prevalence rates of PTSD in HIV^+ samples (approximately 30-50%) indicate the importance of further research in this topic area (Boarts *et al.*, 2006; Kelly *et al.*, 1998; Safren *et al.*, 2003). As with studies concentrating on depression, the literature describes disparate findings, which are

usually explained by methodological issues such as insufficient follow-up time (Boarts *et al.*, 2006; Evans *et al.*, 2002; Kopnisky *et al.*, 2004).

Despite these difficulties, there is evidence amongst the literature that PTSD has a deleterious effect on HIV disease (Kelly *et al.*, 1998; Leserman, 2003; Reilly *et al.*, 2009). Exposure to traumatic events has been demonstrated to impact on $CD4^+/CD8^+$ T-cell ratio at one-year follow-up (Leserman, 2003), with PTSD-associated death anxiety correlating strongly with HIV symptom experience (Safren *et al.*, 2003). An interesting finding to emerge is that those with PTSD, unlike those with chronic anxiety, have lower morning salivary cortisol and less diurnal cortisol difference than those without symptoms of PTSD (Delahanty *et al.*, 2004). Furthermore, this study also revealed that those with PTSD had higher levels of $CD4^+$ T-cells, which is suggested to be due to the stunted cortisol response as cortisol increase is associated with $CD4^+$ T-cell decline (Chida & Vedhara, 2009; Delahanty, Bogart & Figler, 2004; Kopnisky *et al.*, 2004; Leserman, 2003). However, despite the higher $CD4^+$ T-cell counts being observed in PTSD HIV^+ samples, there is also an association between PTSD and higher (or detectable) viral load (VL) (Boarts *et al.*, 2006). This effect was more strongly observed in a population of participants who were suffering from PTSD as a direct cause of a natural disaster, as opposed to other samples whose origins of PTSD traced back to HIV or related issues (Reilly *et al.*, 2009). Using HIV^+ survivors of hurricane Katrina in New Orleans, Reilly and colleagues followed participants for two years post-event and found participants with PTSD had detectable VL levels at both the one- and two-year follow-up junctures, and also documented a decrease in $CD4^+$ levels at the two-year follow-up (Reilly *et al.*, 2009) suggesting that PTSD in reaction to non-illness based situations could elicit a stronger response. The literature presents an overriding association between PTSD and medication adherence, with some study groups suggesting that PTSD only has an indirect affect on HIV disease course via adherence (Boarts *et al.*, 2006; Delahanty *et al.*, 2004). It is therefore possible that in HIV-related PTSD that adhering to the medication regimens may serve as a reminder to their serostatus and therefore result in more distress (Delahanty *et al.*, 2004). This is a factor that may not be observed in groups of HIV patients with PTSD that is unrelated to their diagnosis (e.g. Reilly *et al.*, 2009). Psychosocial impact on medication adherence will be discussed later.

Individual Differences

Psychological morbidity constitutes a significant problem for those with HIV, but there are still issues concerning variation in these findings (Evans *et al.*, 2002; Kopnisky *et al.*, 2004). One way of understanding these disparities is by examining the individual differences in areas such as coping, personality and disposition, which potentially could explain some of the variance within and without psychological morbidity data (Temoshok *et al.*, 2008a, 2008b).

Coping Styles

The analysis of coping style as a factor in itself is very difficult, particularly in the health psychology setting, due to the interactions that coping style has with other factors such as personality, disposition, psychological morbidity, social support structures and patterns of behaviour (Balbin *et al.*, 1999; Vassend & Eskild, 1998). There is also the issue that different coping styles may be effective at specific times in a progressive illness, but not in others, which means that temporally consistent coping styles could elicit both positive and negative effects (in either order) (Ironson *et al.*, 2005b; Temoshok *et al.*, 2008a; Vassend & Eskild, 1998). Nonetheless, coping styles have been shown to produce some of the strongest host relationships to HIV disease progression amongst the literature of psychosocial prognostic indicators (Chida & Vedhara, 2009).

One of the most studied styles of coping is that of Type C coping, which is defined as a difficulty in recognising, processing and communicating those physiological and psychological cues of stress or emotional difficulty (Solano *et al.*, 2002; Temoshok *et al.*, 2008a; 2008b). Type C coping is usually characterised as a personality factor, but the current discussion relates exclusively to the coping styles characterised by Type C personality. Historically, Type C coping has been used to assess prognostics in cancer, but has recently been applied to HIV (Solano *et al.*, 2002; Temoshok *et al.*, 2008a; 2008b). Stronger Type C coping has been associated with higher interleukin (IL) -6 production and increased disease progression in HIV (Temoshok *et al.*, 2008a; 2008b). This observed increase in IL-6 production has a direct impact on the replication of HIV, as it is a proinflammatory cytokine that upregulates the expression of CCR5 coreceptor on human immune cells, which facilitates R5-tropic HIV viruses in binding to and infiltrating healthy cells (Langford *et al.*, 2007; Temoshok *et al.*,

2008a) An increased presence of IL-6 also increases the apoptosis (programmed cell-death) of $CD8^+$ T-cells, meaning that IL-6 and other proinflammatory cytokines have an HIV facilitating action irrespective of the HIV virus strain (both R5- and X4-tropic) (Langford *et al.*, 2007).

However, as aforementioned, there may be temporal effects in this relationship (Solano *et al.*, 2002; Temoshok *et al.*, 2008a). One study, which observed the relationship between Type C coping and HIV disease progression, categorised their participants into their CDC classification of HIV disease stages (Solano *et al.*, 2002). With this categorisation, the deleterious effect between higher levels of Type C coping and HIV progression was only observed in those with more advanced HIV disease (CDC category A2; with $CD4^+$ T-cell counts between 200 and 499 cells/mm^3), and not in those within the prior categorisation (CDC category A1; with $CD4^+$ T-cell counts over 500 cells/mm^3) (Solano *et al.*, 2002). This finding suggests that whilst $CD4^+$ T-cell counts are over 500 cells/mm^3, and physiological HIV symptoms are at a minimum, Type C coping is not particularly destructive. However, once $CD4^+$ T-cell levels drop, and symptoms develop and feelings of illness increase, this coping pattern, which is characterised by a lack of ability to process those feelings, becomes detrimental (Solano *et al.*, 2002; Temoshok *et al.*, 2008a).

Further coping styles that have been found to impact on HIV disease progression are those which are reactionary to HIV diagnosis; posttraumatic growth (Milam, 2006) and increases in spirituality or religiousness (Ironson *et al.,* 2006). Both posttraumatic growth and an increase in spirituality/religiousness have shown to be protective factors for $CD4^+$ T-cell counts in HIV (Ironson *et al.,* 2006; Milam, 2006), with a decrease in spirituality or religiousness post-diagnosis suggested to increase the speed of $CD4^+$ T-cell loss by four and a half times (Ironson *et al.,* 2006). Those other coping styles which have been suggested to negatively impact on HIV disease are passive coping, which has been shown to increase the reports of AIDS symptoms in HIV^+ youth (Stein & Rotherum-Borus, 2004); the practice of "venting" (immediately expressing emotions without processing them) which has been suggested to increase the report of HIV symptoms in adults (Ashton *et al.*, 2005); and denial or avoidant coping which has associations with increased viral load and decreased $CD4^+$ T-cell counts prospectively (Ironson *et al.*, 2005b). A coping style termed "planful problem solving" (i.e. that which requires cognitive processing of problems, and planning of resolutions) has been observed to be a protective factor in HIV disease, with a slower

clinical progression than those who do not adopt this coping style (Vassend & Eskild, 1998; Vassend *et al*., 1997). Adaptive coping is also a factor involved in attenuating disease progression in HIV. A study involving long-term survivors (those with a symptom-defined AIDS status for over four years) showed adaptive coping to increase NK cell cytotoxicity and decrease VL (Soloman *et al.*, 2002).

Further support for these findings comes from intervention studies that have been targeted to ameliorate emotional expression of stress and grievances. One such study employed a brief intervention based on written emotional expression. This required randomised participants to write for half an hour a day for four consecutive days, with the experimental group being asked to write about their most emotional or traumatic life experiences and the control group being asked to write about a neutral topic (Petrie *et al.*, 2004). Six months after this intervention, an increase in $CD4^+$ T-cell count was still being observed in those from the experimental group, which considering the brief nature of the intervention is a strong support for the effects of active coping and emotional disclosure (Petrie *et al.*, 2004).

Personality and Disposition

Personality as a psychological construct has been applied to the understanding of health and disease across many patient groups (Kubzansky *et al.*, 2009). As explained above, Type C coping is an aspect of Type C personality and has been explored in terms of its effects on both HIV and cancer (Ironson *et al.*, 2008; Solano *et al.*, 2002; Temoshok *et al.*, 2008a; 2008b). In basic terms personality, as a construct, influences behaviour, beliefs and cognitions, coping mechanisms, information processing and contextual interpretation, and decision-making (Ironson *et al.*, 2008). As such, personality (and its composites) is an important host factor in understanding the individual differences of HIV disease progression.

Examination of personality traits and HIV progression is still a relatively new field, but the initial findings appear to be promising. A one-year study conducted with HIV patients looked at the trait of conscientiousness (e.g. competence, order, dutifulness, achievement striving, self-discipline and deliberation) and found that it was related to both $CD4^+$ T-cell count and VL measures longitudinally (O'Cleirigh *et al.*, 2007). Those participants who scored high in levels of conscientiousness were observed to experience a mean increase of 59 in $CD4^+$ T-cell counts and a mean decrease of 1,727

in VL, compared to those low in conscientiousness who experienced a mean decrease in $CD4^+$ T-cells of 27 counts and a mean increase in VL of 13,000 (O'Cleirigh *et al.*, 2007). This finding is particularly impressive given that in HIV, even if the patient is using HAART, a steady decline in $CD4^+$ T-cell count is usually observed over time (O'Cleirigh *et al.*, 2007). A follow-up to this study was conducted a year later, using more personality traits to evaluate the effect of broader aspects of personality on HIV disease progression (Ironson *et al.*, 2008). This study observed similar trends in conscientiousness, but also expanded to show both openness and extraversion, and performed a four-year follow-up to establish longer-term correlates of personality (Ironson *et al.*, 2008). Both openness and extraversion were associated with an increase in $CD4^+$ T-cell count over four years, and variations in conscientiousness were shown to predict the increase in VL normally observed over such a time period (Ironson *et al.*, 2008). The authors explained these results by surmising that those who were high in extraversion would be more likely to sustain their social relationships and those high in openness would be more optimistic (Ironson *et al.*, 2008).

Further personality factors that have received attention in the research community are optimism and pessimism. Optimism and pessimism are dispositional factors, which are characterised as the ability to create positive or negative interpretations to life events and the maintenance or lack of hope in situations of adversity (Ironson *et al.*, 2005a; Milam *et al.*, 2004). Optimism has been associated with increases in $CD4^+$ T-cell counts over time in those with HIV (Ironson *et al.*, 2005a; Milam *et al.*, 2004). However, this relationship is suggested to be curvilinear as those with moderate levels of optimism have higher $CD4^+$ T-cell counts than both low and high optimism groups (Milam *et al.*, 2004). This curvilinear relationship between optimism and $CD4^+$ T-cell counts suggests that there is a point where optimism becomes unrealistic and ultimately detrimental. In effect, those with very high expectations that are eventually not realised will experience more stress than those with lower expectations that are realised (Milam *et al.*, 2004; Temoshok *et al.*, 2008a). Therefore optimism must be metered with a sense of realistic expectation to be effective in promoting health in HIV. Those with above average levels of optimism have been shown to experience above average increases in $CD4^+$ T-cell counts and slower VL increase, whereas those with lower levels of optimism see above average decreases in $CD4^+$ T-cells and higher VL increase over a two year follow-up period (Ironson *et al.*, 2005a). However

a study examining both optimism and pessimism found no such relationship for optimism and VL, in fact optimism was only related to $CD4^+$ T-cell count and pessimism was related to VL only (Milam *et al.*, 2004).

As the impact of personality factors on HIV is still a relatively new field, no research to date has examined the possible mechanisms that underlie the relationship. However, it stands to reason that as personality factors have associated behaviours and cognitions that relate to them, it is very likely that these may serve as facilitative factors (Ironson *et al.*, 2005a). However, in their study of the effects of different levels of optimism and pessimism on HIV prognosis, Milam *et al.* observed no differential relationships with health behaviours on HAART adherence (Milam *et al.*, 2004), which suggests there should be other underlying causes for this interaction. It is also quite possible that pessimism may be a factor of depression, as it is a characteristic of depressed affect, and it should be noted that depression has been suggested to be a mediator in the findings of Ironson *et al.,* who examined varying levels of optimism and its effects on $CD4^+$ and VL (Ironson *et al.*, 2005a).

SOCIAL FACTORS

The host social elements of being HIV^+ are multi-factorial. A diagnosis of HIV not only has health consequences, but due to its communicability and related stigma, it leads to significant social change for the patient (Pakenham & Rinaldis, 2001; Pryor *et al.*, 1999; Ullrich *et al.*, 2004). Social resources can help to manage the changes involved in an HIV diagnosis as well as facilitating adaptive coping to new challenges and difficulties (Burgoyne, 2005). Two of the main social factors involved in disease progression are social support and social identity

Social Support

Social support is one of the most well established social factors responsible for impacting on disease progression and is defined as resources for emotional, practical and informational problems from family, friends, partners or organisations (Ashton *et al.*, 2005; Young *et al.*, 2004). The examination of social support in HIV has classically been regarding issues such as health-related quality of life, but has more recently been reviewed in regard to its impact on disease progression (Burgoyne,

2005). As previously discussed, prevalence rates of psychological morbidity in HIV^+ patients are very high, but these psychological difficulties can indeed be buffered by social factors, provided that social support is satisfactory and accessible (Ashton *et al.*, 2005; Young *et al.*, 2004). The literature surrounding this relationship has classically been more related to social support as this buffering factor for distress, but is beginning to be examined as a risk factor in itself (Burgoyne, 2005).

The data concerning this relationship has also been contradictory, with some studies outlining a high level of social support as a protective factor and some as a damaging factor (Balbin *et al.*, 1999; Burgoyne, 2005; Chida & Vedhara, 2009). Studies that have shown a relationship between higher social support and decreased HIV progression have observed this relationship in terms of suppressed VL (Burgoyne, 2005). Another perspective of viewing this relationship has been in the form of perceived satisfaction with social support, which in itself provides a more salient measurement for such a highly subjective construct. Subjective satisfaction with social support has been found to predict HIV symptoms at one year follow-up, with lower levels of social support satisfaction being related to higher levels of HIV symptom experience (Ashton *et al.*, 2005) and faster progression through CDC stages (Leserman *et al.*, 2002).

Social inhibition has also been examined as a component of social support, with those with higher levels of social inhibition displaying higher baseline levels of VL and poorer immunologic responses to HAART prospectively (Cole *et al.*, 2003). Moreover, this study related social inhibition to autonomic nervous system (ANS) activity, concluding that ANS activity is a mechanistic factor between social inhibition and HIV immunologic outcome and is independent of a variety of psychosocial, biological and HIV-related variables (Cole *et al.*, 2003).

A further facet of social support researched in relation to HIV prognostics is affects of a stable partnership (Young *et al.*, 2004). Stable partnership can be a protective factor in HIV disease progression in terms of immunologic and physiologic HIV-related outcomes, but was also reported to decline with increasing disease (Young *et al.*, 2004).

Several studies have, however, failed to support these findings, and have found either no discernable relationship between social support and HIV pathogenesis (Balbin *et al.*, 1999) or have found trends in the opposite direction, with findings of higher baseline social support predicting faster $CD4^+$ T-cell decline (Ironson *et al.*, 2005b).

Suggested reasons for this harmful relationship have been postulated as occurring via behavioural and social mechanisms, whereby good social networks foster either risky health behaviours or repeated exposure to traumatic circumstances such as AIDS-related bereavement or caregiving (Miller & Cole, 1998). A recent meta-analysis of psychosocial prognostic factors in HIV concluded that there is currently not enough evidence, either in strength or number, to support a direct relationship between social support and HIV prognosis (Chida & Vedhara, 2009). This is proposed to be due to a number of factors, most notably the conceptualisation of social support, its component factors and their relevance to the individual (Chida & Vedhara, 2009). The authors also raise an interesting point which requires further study; namely that women may benefit from social support more than men - and propose that further research be conducted with female samples to assess the relationship (Chida & Vedhara, 2009). There is also the issue that social support in itself may indeed be affected by HIV, as the disease worsens and the individual feels more ill and requires more time at rest, so social relationships may weaken or dissipate (Burgoyne, 2005). It is therefore difficult to review the effects of social support on HIV prognosis if the disease has developed to the point where the patient's social life is impacted significantly, and this could account for some of the discrepant findings in the literature.

Social Identity and Inhibition

How individuals relate to their social systems can be a more difficult issue win HIV patients (Cole *et al.*, 1997; Fekete *et al.*, 2009). One of the highest proportions of HIV infections globally is transmission amongst men who have sex with men (UNAIDS, 2010), which makes homosexual HIV^+ populations doubly sensitive to social issues. Social identity in HIV populations refers to the disclosure of HIV status and homosexual identity (Cole *et al.*, 1997). The act of disclosing homosexuality (or "coming out") is a psychosocial milestone for gay people: with the possibility of this disclosure being met with hostility, disappointment, prejudice or other negative attitudes and behaviours (Cole *et al.*, 1997; Ullrich *et al.*, 2003). Equally the disclosure of an HIV^+ status has the potential to be met by similar negative social reactions due to the stigma heavily laden on the condition since its discovery (Fekete *et al.*, 2009; Pryor *et al.*, 1999). Whilst some may choose to disclose these issues to their immediate social network, and sometimes beyond, others may choose to conceal them from even their closest family and friends, which can result in this type of

inhibitory behaviour "spilling" over to other aspects of their lives (Eisenberger *et al.*, 2003; Ullrich *et al.*, 2003). Social inhibition has been associated with increased ANS activation; which, in turn, was also associated with elevated VL and decreased response to HIV treatment (Cole *et al.*, 2003).

Concealment of Sexual Identity

The concealment of homosexuality from one's social network has been associated with faster HIV disease progression (Cole *et al.*, 1997; Ullrich, *et al.*, 2003). Additionally, the relationship between concealment and disease progression also appears to be influenced by satisfaction with social support (Ullrich *et al.*, 2003). Further, the effects of concealment may be less pronounced amongst those who are rejection-sensitive (Cole *et al.*, 1997). This suggests that although there is a relationship between the concealment of homosexual identity and faster HIV disease progression, it is highly likely to be relative to that individual's social and personal context. The data suggest that in those who are less sensitive to rejection and who have higher levels of self-regard and satisfaction with social support, the concealment of homosexuality would be a damaging factor for HIV prognosis (Cole *et al.*, 1997; Ullrich *et al.*, 2003; 2004). However, concealment of homosexuality can serve as a protective factor in those with lower levels of self-regard and who are more rejection-sensitive, as they are not risking negative social consequences, which they may be less equipped to deal with (Cole *et al.*, 1997; Ullrich *et al.*, 2004).

Concealment of HIV Status

Two studies to date have assessed the impact of disclosing HIV^+ status on the progress of the illness (Fekete *et al.*, 2009; Sherman *et al.*, 2000). One study of HIV^+ children categorised them into those who had recently disclosed their HIV status to their friends, those who had previously disclosed and those who had not disclosed and compared their $CD4^+$ T-cell percentage decline over one year (Sherman *et al.*, 2000). Amongst the children, those who had disclosed their HIV status showed a significant increase in $CD4^+$ T-cell percentage over one year (Sherman *et al.*, 2000). In contrast, those who had not previously disclosed their HIV status did not experience $CD4^+$ T-cell increase (Sherman *et al.*, 2000). The percentage increase was greater in those recently disclosing serostatus than that observed in those who had previously

disclosed, which suggests that the most pronounced effects of this disclosure are immediate, but that they can endure (Sherman *et al.*, 2000).

In adults HIV status disclosure has also been associated with a slower disease progression and has also been described culturally (Fekete *et al.*, 2009). In a study analysing the differences in $CD4^+$ T-cells and VL amongst men who divulged their HIV serostatus to their mothers, these changes were assessed differentially in Caucasian and Latino cultures and across those with different levels of perceived HIV-related family support (Fekete *et al.*, 2009). The results showed that amongst Caucasian men who disclosed their HIV status to their mothers, those who had higher levels of perceived support had lower VL and higher $CD4^+$ T-cells than did those who reported low perceived levels of support (Fekete *et al.*, 2009). Inversely, Latino men who disclosed their serostatus to their mothers only showed an improvement in HIV progression markers (VL) if they reported low levels of perceived support, whereas those who reported high levels of support showed no relationship between disclosure and $CD4^+$ T-cell count or VL (Fekete, 2009). Equally no associations could be found amongst men who disclosed their HIV status to their fathers (Fekete, 2009). Caucasian men only display a difference in prognostic markers ($CD4^+$ T-cell count and VL) if they perceive higher levels of HIV-specific family support, and do not appear to be at a detriment if they have lower levels of support (Fekete, 2009). However, Latino men do not experience any increase in HIV suppression even if they report high levels of perceived support but rather do appear to be at a detriment if their disclosure results in low levels of HIV-related family support (Fekete, 2009). These findings suggest that familial disinhibition is culturally relevant and further differences could be observed across other racial and ethnic demographics. It also appears to be relevant to the individual in terms of how much support they receive once they have made this disclosure.

On a more general level of social inhibition, a recent study using interviews with HIV^+ women has described an association between the use of language related to inhibition when undergoing a "coping with HIV" interview, and $CD4^+$ T-cells. The study revealed that those who display higher levels of inhibition have lower $CD4^+$ T-cell counts (Eisenberger *et al.*, 2003). Even when controlling for a broad variety of physiological, psychological and behavioural covariables, levels of social inhibition still contributed to 8% of the variance observed in $CD4^+$ T-cell count across the

participants (Eisenberger *et al.*, 2003). This observation was still maintained when accounting for emotional expression, which suggests that the relationship between inhibition and HIV suppression is not influenced by emotional expression as one might expect. It is therefore possible that the deleterious effects of inhibition are exerted through other behavioural or interpersonal mechanisms such as decreased socialisation, increased stress due to the withholding of pertinent personal information or decreased self-care due to the denial or suppression of HIV-relevant physiological cues.

BEHAVIOURAL FACTORS

The behaviours of the individual that impact upon HIV progression can exist independently or can be resultant from the previously discussed psychological or social factors (see figure three). The three main areas in which behavioural factors decrease HIV suppression are HAART medication adherence, the abuse or dependence of alcohol or addictive substances and health behaviours. Whilst these behaviours can serve as mechanisms from other areas of psychosocial prognostic indicators and disease progression, they are concepts within themselves, and their prevalence and predispositions have been studied well in recent years.

HAART Adherence

Since its widespread availability in the late 1990s, HAART use has been associated with an estimated two-thirds decrease in mortality amongst those with end-stage HIV disease ($CD4^+$ T-cells<100 copies/mm^3) (Weller & Williams, 2001). The combination of drugs that act on various stages of the virus' replication has resulted in the ability to suppress HIV viraemia to near undetectable levels (the "gold standard" of combination therapy) (Deeks, 2006). With this optimum suppression, the immune system can then reconstitute and function to its highest capacity (Deeks, 2006; Weller & Williams, 2001). The development and widespread availability of HAART now means that HIV can be considered more a chronic illness rather than a terminal one (Parruti *et al.*, 2006; Pence, 2009). This means understanding the reasons why patients do not adhere to their medication is an important issue in treating the global problem of HIV.

Those who do not adhere to their HAART medication regimes may do so for several reasons, and can be loosely categorised into three groups: erratic, unwitting and intentional (Van Servellen *et al.*, 2002). Those who are erratic non-adherents are characterised by circumstantial barriers to adherence. Although they understand how and why to take their medications there may be situations in which they miss doses (for example if they forget, run out or are too busy) (Van Servellan *et al.*, 2002). Unwitting non-adherents are those people who have a poor understanding of the schedule of their medication regimes and intentional non-adherents are those who actively choose not to adhere (Van Servellen *et al.*, 2002). Intentional non-adherence is an issue particularly pertinent to HIV not just because adherence is required for optimum viral suppression and prevention of viral mutation (to a drug-resistant state), but because there is a heavy pill-burden in HAART with drugs which elicit numerous side-effects and serve as a constant reminder of illness (Langford *et al.*, 2007; Pence, 2009; Parruti *et al.*, 2006; Van Servellan *et al.*, 2002).

Optimum adherence to HAART has been related first and foremost with good tolerance of the drugs being taken, with the experience of side-effects from the medications being cited as the most common reason for non-adherence (Parruti *et al.*, 2006; Applebaum *et al.*, 2009). Stressful life events and depressive symptoms have also been observed to have an inverse relationship with medication adherence (Leserman *et al.*, 2008). Married people are reported to be twice as likely to retain good levels of adherence in comparison to single people, irrespective of the HIV serostatus of the spouse (Parruti *et al.*, 2006). Psychosocial predictors of poor adherence include the experience of trauma, depression, substance abuse, homelessness and stress (Leserman *et al.*, 2008; Mugavero *et al.*, 2007; Parruti *et al.*, 2006; Uldall *et al.*, 2004). On a more global scale, access to health care and the attitude with which health professionals treat patients also has a predictive significance on medication adherence (Schneider *et al.*, 2004; Van Servellen *et al.*, 2002).

Alcohol or Addictive Substance Abuse & Dependence

Substance use and abuse is commonly found amongst HIV^+ populations and constitutes a significant problem in relation to HIV treatment (Cabral, 2006; Fiellin, 2004; Uldall *et al.*, 2004). The use or abuse of addictive substances carries with it an

inherent impact upon HIV progress in the form of medication adherence, as intoxication is associated with negligent behaviour and the primary prioritisation of maintaining the addictive behaviour (Gore-Felton & Koopman, 2008). However, once someone has received treatment for substance dependence they may still experience faster HIV disease progression and greater adherence problems than patients who have never had dependency problems, which is thought to be attributable to the social and psychiatric circumstances of such populations (Spire *et al.*, 2007). The routes of administration of certain drugs can also have an impact on HIV. Those drugs that are commonly injected (e.g. opiates, cocaine, and methamphetamine) not only facilitate the spread HIV, but also other blood-borne pathogens (Donahoe & Vlahov, 1998). Coinfection with other pathogens may impede the immune system, and can also create multiple HIV exposures in an individual with the possibilities of multiple viral subtype infections, which results in more virulent HIV infection and more complicated treatment (Donahoe & Vlahov, 1998; Gore-Felton & Koopman, 2008).
Longitudinal research by the United States CDC has suggested that injection drug users (IDU) as a group show extraordinarily fast conversion from HIV diagnosis to AIDS, with around 40% IDU experiencing this transition in just 12 months (Grigoryan *et al.*, 2009). The introduction of some of these substances to the body can also change the general functioning of the immune system and can interact with HAART (Fiellin, 2004; Gore-Felton & Koopman, 2008). Whilst research into the effects of drugs of abuse on HIV progression is still ongoing, only that which has provided consistent support throughout the literature will be presented here. Other avenues of interest that are still being debated are marijuana and tobacco.

Alcohol

Alcohol consumption has been associated with enhanced HIV viraemia, decreased HAART uptake and adherence, and increased cerebral atrophy (which has been related to the neuropathogenensis of HIV) (Fiellin, 2004; Pfefferbaum *et al.*, 2007; Samet *et al.*, 2007). Some studies have argued that alcohol dependency or abuse has no direct biological action on HIV, and only results in accelerated pathogenesis by its behavioural effects on adherence (Chander *et al.*, 2006). However, the wealth of the literature on this subject suggests that there are direct biological consequences of alcohol abuse in HIV disease. Physiologically, alcohol is supposed to have additive effects to HIV in immunodegeneration and neurological damage, over and above that

observed for either condition in exclusion (Fiellin, 2004; Pfefferbaum *et al.*, 2007; Samet *et al.*, 2007).

In trying to extract the biological from the behavioural, one study examined heavy alcohol use and its effects on $CD4^+$ T-cell count and VL in HIV^+ participants (Samet *et al.*, 2007). In this study, heavy alcohol use still correlated with lower VL in those taking HAART once adherence was controlled for, and correlated with lower $CD4^+$ T-cell counts but only in those who were not taking HAART (Samet *et al.*, 2007). These findings suggest that although there are some effects that may be attributable to adherence, alcohol abuse still exerts a negative effect on HIV pathogenesis. In light of the fact that alcohol abuse correlated with VL in HAART users and not in non-HAART users, it is possible that alcohol use has an interactive effect on HAART medications (which are primarily responsible for curtailing VL).

Amphetamines

The use of methamphetamines can be found quite commonly amongst samples of HIV^+ men who have sex with men (MSM) due to the social associations of methamphetamine use in the MSM demographic, and its consequential effects on high risk sexual behaviour (Marquez *et al.*, 2009; Nakamura *et al.*, 2009). The use of methamphetamine has been associated with the dysfunction and neuroadaptation of the dopamine and serotonin pathway network systems within the brain, which is known to cause immunomodulation and compromise the blood brain barrier, facilitating neuro-infection of HIV and therefore overall disease progression (Cabral, 2006; Chang *et al.*, 2005; Mahajan *et al.*, 2008). As with alcohol, there is a suggestion that methamphetamine use has an additive effect to HIV in the degradation of brain matter volumes, including the compromised integrity of the blood brain barrier, both of which contribute to the neuropathogenesis of HIV (Chang *et al.*, 2005; Mahajan *et al.*, 2008).

Cocaine

Cocaine and crack-cocaine are another sub-section of drugs of abuse that have been suggested to impact upon the progression of HIV (Cook *et al.*, 2008; Fiellin, 2004). Cocaine use has been associated with immunomodulation, with changes in T-cell activity and the balance between Th_1 and Th_2 immunity patterns, which may impact on the immune system's ability to control HIV and its associated opportunistic

infections (Cabral, 2006; Fiellin, 2004). Crack cocaine is commonly smoked, and there have been strong associations found between the smoking of crack and the incidence of pulmonary-associated HIV-related diseases, which may contribute to the associations between cocaine use and HIV disease progression (Webber *et al*., 1999).

As with other illicit drugs whose use has been investigated in relation to the progression of HIV, it is broadly understood that cocaine and crack-cocaine use exerts its effect on pathogenesis by interfering with HAART adherence (Baum *et al.*, 2009; Fiellin, 2004; Webber *et al.*, 1999). However, in recent years more research has been conducted to examine these effects whilst controlling for adherence, and crack-cocaine has been independently associated with markers of disease progression (Baum *et al.*, 2009; Cook *et al.*, 2008). In a longitudinal study examining the effects on disease progression in crack-using women, use was demonstrated to relate to increased HIV viraemia and immune deterioration, the diagnosis of an AIDS-defining condition and AIDS-related mortality independent of HAART use or adherence (Cook *et al.*, 2008). In a recent study, Baum *et al.* have demonstrated not only an impact of crack-cocaine use on VL, but also on $CD4^+$ T-cell count, again independent of HAART use or adherence over a prospective data collection period of 30 months (Baum *et al.*, 2009).

Opioids

Research into the effects of opioid use on HIV progression is relatively sparse and inconclusive, which is surprising considering the strong relationship between injection drug use and HIV infection (Fiellin, 2004). Much is known about the effects of opioids on the immune system, yet comparatively little is understood about their effects in HIV^+ patients. This is supposedly due to the difficulties in isolating opiate use as a predictor from other issues such as adherence, polydrug use and toxic poisoning by excipient substances (Donahoe & Vlahov, 1998; Roy *et al.*, 2006). One of the most pressing issues in HIV^+ opioid addiction is that of the availability of HAART, as many IDU may not be provided this treatment until they are receiving treatment for their addiction (Fiellin, 2004; Spire *et al.*, 2007). That said, it has been suggested that opiate replacement therapy (with agents such as methadone or buprenorphine) is associated with better adherence in opiate addicted samples (Spire *et al.*, 2007; Uldall *et al.*, 2004).

HIV aside, the use of opioids is known to decrease the volume of both the thymus and spleen; secondary lymphoid organs vital to the production of lymphocytes and the differentiation of immature T-cells to $CD4^+$ T-helper cells or $CD8^+$ cytotoxic T-cells (Cabral, 2006; Roy & Loh, 1996; Roy *et al.*, 2006). Chronic opioid use is also suggested to affect NK cell function and cytotoxicity, as well as suppressing cellular immunity (Cabral, 2006; Roy & Loh, 1996; Roy *et al.*, 2006). Evidence from *in vitro* research suggests that opioids upregulate HIV (Donahoe & Vlahov, 1998; Gore-Felton & Koopman, 2008). However, some recent research into Simian Immunodeficiency Virus (SIV) has suggested that opioid exposure helps to slow down the progression of SIV (Donahoe *et al.*, 2009). It is possible that these differences observed in the simian model may have occurred through the different levels of stress. The authors posit that as the opiate dependence of the laboratory monkeys was well maintained, that they may not experience the physiological and psychological stress that human opiate dependents may do (Donahoe *et al.*, 2009). Further, it is this stress that elicits the activation of latently infected HIV cells, thereby increasing circulating virus.

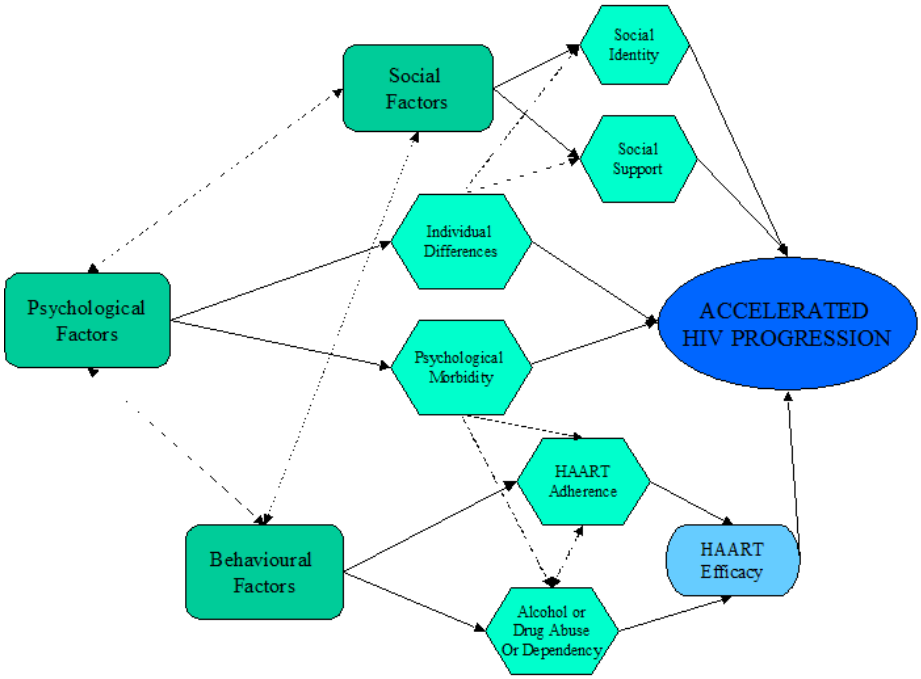

Figure 3. Host psychosocial contributers to accelerated HIV progression

PNI PATHWAYS OF DIFFERENTIAL HIV PROGRESSION

Whilst the mechanisms from one phenomenon to another can be explained through behaviour (i.e. social support to HIV acceleration, via HAART adherence (see figure three), there are some direct biological pathways that allow psychological states to exert effect upon immune system actions. These pathways serve to explain some of the differences observed from person to person in HIV disease, although more research is required in order to fully understand these mechanisms.

Recent clinical evidence has sought to elucidate the mechanisms that may serve to explain the relationship between psychological morbidities and increased HIV progression. NK cells and $CD8^+$ (cytotoxic) T-lymphocytes are two of the leading cells impacted by the depression-disease progression relationship in HIV, with a relatively decreased number of NK cells being observed in HIV^+ patients with depression compared to those without (Alciati *et al.*, 2007; Evans *et al.*, 2002; Greeson *et al.*, 2008; Ironson *et al.*, 2005a; Pence, 2009). In HAART-naïve patients, it has been suggested that major depression only relates to NK cell count and percentage, in contrast to non-depressed matched HIV^+ controls (Alciati *et al.*, 2001). This suggests that the findings of depression's impact upon $CD8^+$ T-cells observed in HAART using samples may be due to the modulation of HAART action by the neurophysiological consequences of depression. One study conducted by Evans and colleagues (2002) describes a functional alteration in lymphocyte activity, with lower levels of NK cell activity and higher levels of activated $CD8^+$ T-lymphocytes being observed in HIV^+ women with symptoms of depression and anxiety (Evans *et al.*, 2002).

Other theories include modulation of the sympathetic nervous system (SNS) in depression and stress, with the biogenic amine Noradrenaline being cited as a likely perpetrator (Chida & Vedhara, 2009; Cole, 2008; Greeson *et al.*, 2008; Hassan & Douglas, 1990; Kopnisky *et al.*, 2004; Leserman, 2003). Receptors for this neurotransmitter have been found on the cell surface of many cell classes within the immune system and can alter the way in which the immune system functions at rest or in response to infection (Besedovsky & Del Ray, 2007; Taub, 2008).

A recent suggestion is that Substance P (SP) may be involved as a mechanism by which depression (and other psychosocial factors) may impact in disease progression. Evidence for this postulation comes from observations of high levels of SP in the plasma of HIV^+ patients, the effectiveness of SP receptor antagonists in treating

depression, and the role of SP in immunoregulation (Cole, 2008; Ho & Douglas, 2004; Kopnisky *et al.*, 2003; Leserman, 2003; Li *et al.*, 2001). It is proposed that SP facilitates HIV replication through several means. Firstly, SP elicits the activation of NF-κB (a genetic transcription factor), which activates CCR5 expression on immune cells allowing R5-tropic HIV virus more receptors to target for cell invasion (Li *et al.*, 2001). Secondly, SP stimulates the release of pro-inflammatory cytokines, including Tumor Necrosis Factor (TNF)-α, which is known to facilitate the upregulation of HIV-1 infection in T-cells and monocytes (Ho & Douglas, 2004; Kopnisky *et al.*, 2004; Li *et al.*, 2001). Thirdly, SP has been demonstrated to activate latent HIV-1 virus in infected cell lines, most notably T-lymphocytes, thereby exponentially increasing host VL (Ho & Douglas, 2004; Li *et al.*, 2001). Evidence for this direct link comes in the form of exerting SP receptor antagonists on the relevant cells, where the effect of SP on HIV-1 cells is abrogated as binding of SP is blocked by the antagonist (Ho & Douglas, 2004; Kopnisky *et al.*, 2004; Li *et al.*, 2001). Moreover, HIV has been suggested to increase serum levels of SP, thereby creating what is termed a *feed-forward* cycle where both HIV and SP promote each other symbiotically (Ho *et al.*, 2002; Kopnisky *et al.*,, 2004; Leserman, 2003).

Anxiety's detrimental effects on HIV are thought to be driven by the increased activity of the Hypothalamic-Pituitary-Adrenal (HPA) axis, and production of cortisol (Chida & Vedhara, 2009; Cole, 2008; Ironson *et al.*, 2005b; Kopnisky *et al.*, 2004; Leserman, 2003; Leserman *et al.*, 2002). The stress-induced over production of cortisol is thought to affect HIV pathogenesis by switching immune response patterns from Th_1 to Th_2 (Chida & Vedhara, 2009; Kopnisky *et al.*, 2004; Leserman, 2003). This switch diminishes the attack response to HIV and accelerates apoptosis of antiviral immune cells, and possibly also accelerates HIV replication further as there have been some suggestions that HIV-infected cell lines respond to cortisol and dexamethasone (Chida & Vedhara, 2009; Kopnisky *et al.*, 2004; Leserman, 2003). However, research into the effects of glucocorticoids on HIV has suggested that they may actually suppress HIV replication by bonding to and suppressing long-terminal repeat activity (that which enables the virus to enter a host cell) (Kino *et al.*, 2000), so this requires more research.

CONCLUSION

The ways in which psychosocial aspects of the individual impact upon HIV progression are multifactorial and often interlinked (see figure three). Although we understand much about how certain conditions affect HIV disease, there is still much we do not know.

Intervention studies have afforded us the opportunity to assess our ability to ameliorate or reverse some of the adverse effects of psychosocial circumstance on HIV, and have underlined the importance of psychosocial support during illness. For example, the intervention for emotional expression by Petrie and colleagues delineated the longitudinal efficacy of simple, brief strategies, which can be employed universally to HIV patients due to their simplicity (Petrie *et al.*, 2004). Such interventions also aid in improving the patients' experiencing of living with their illness, as well as trying to prolong their lives. The present identified factors are all fraught with theoretical contention, and it is immediately apparent that observational breadth and good covariable control are essential to furthering our knowledge. However, there are some conditions under which we may not be able to accurately isolate prognostic indicators from extraneous variables, such as is seen with analysing the effects of drug and alcohol dependence on HIV progression.

In summary, although there is still much to be studied and learned, the literature surrounding the non-viral host-related precedents of HIV progression has allowed us to understand more about the individual variation of HIV disease experience, how to help prolong peoples' lives by targeting these variations and to identify those areas in which our knowledge is still wanting. In the HAART era, whereby patients can live with HIV for much longer than the early years of the epidemic, whilst it is still of vital importance that efforts are made to find a cure, the research into those factors which speed up the progression of the illness helps HIV^+ patients every day and this research should continue.

Chapter 3

Hemispheric Lateralisation and Immune Function: A Systematic Review of Human Research

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Abstract

Past studies examined relationships between hemispheric lateralisation (HL) and immune system functioning. However, there has been no up-dated systematic review of this research area. This article reviews relevant published studies, evaluates study quality and effect sizes. Eleven studies were selected: three revealing a relationship between weaker left hemisphere function and poorer immune function, three describing a relationship between weaker right hemisphere function and stronger immune functioning, and five describing both relationships. Mean effect-size of the studies was $r = 0.536$ (range 0.280-0.866). Collectively, studies point at left-HL and stronger immunity relationships. Limitations, mechanisms and clinical implications are discussed.

INTRODUCTION

Neuroimmunology has uncovered progressively over the last 3 decades the bi-directional manner of communication between the central nervous system (CNS) and immune system (Banks, 2004; Bellinger *et al*., 2008; Besedovsky & del Ray, 1996; Butts & Sternberg, 2008; Ferone *et al.*, 2006; Tracey, 2002; Webster *et al.*, 2002; Wrona, 2006). The immune system communicates with the CNS once an infection has been encountered in the periphery, initiating *sickness behaviour,* providing optimum behavioural conditions to facilitate immune defence and recovery (Banks, 2004; Besedovsky & del Ray, 1996, 2007; Hopkins, 2007; Konsman *et al.*, 2002; Rivest, 2003; Vollmer-Conna *et al.* 2004; Wrona, 2006). One important form of immune-to-brain communication is via the vagal nerve, especially in low levels of peripheral inflammation (Tracey, 2002). The CNS-immune communication is mainly achieved via autonomic and neuroendocrine pathways, (Bellinger *et al.*, 2008; Besedovsky & del Ray, 1996; Butts & Sternberg, 2008; Ferone *et al.*, 2006; Neveu, 1988; Webster *et al.*, 2002; Wrona, 2006). Receptors for various hormones, neuropeptides and neurotransmitters have been found to be expressed on the surface of many immune cell types (Basu & Dasgupta, 2000; Ferone *et al.*, 2006; McKenna *et al.*, 2002; Webster *et al.*, 2002). Organs of the lymphatic system; such as bone marrow, thymus, spleen, mucosal lymphoid tissues and lymph nodes; have been demonstrated to be innervated by autonomic fibers – mainly of the sympathetic division, but parasympathetic involvement has also been described (Bellinger *et al.* 2006; 2008; Quan & Banks, 2007; Tracey, 2002; Wrona 2006). Evidence of CNS-immune relations also comes from neurophysiological observations concerning hemispheric lateralisation (HL), which may help explain some individual differences in brain-immune associations (Neveu, 1988; 1991; 1992). The HL-immune relationship is the topic of this article.

Differential CNS communication with the Immune System – Hemispheric Lateralisation

How the CNS influences the immune system can depend on many factors, one of which is hemispheric lateralisation (HL). The two hemispheres of the human brain have different functional specialisations, and it is well known that one of the hemispheres will be activationally or functionally dominant to the other (Cerqueira *et al.*, 2008; Hugdahl, 2000; Neveu, 1988, 1991, 1992). The two hemispheres of the

brain are known to act differentially upon behaviour, psychiatric and neurological disorders and immunity (Neveu, 1992; Sackeim *et al.*, 1984; Wittling *et al.*, 1998). Experimental studies in animals across the last two decades have demonstrated that unilateral damage or stimulation to either the left or right hemispheres of the brain result in opposite immunological reactions (Moshel *et al.*, 2005; Neveu, 1988, 1991, 1992; Neveu *et al.*, 1991). Damage to the left hemisphere results in the depression of immunological parameters such as T-lymphocyte proliferation, natural killer cell activity (NKCA), IL-2 production and production of Immunoglobulin G antibodies (Goldstein *et al.*, 2002; Neveu, 1988, 1991, 1992, Neveu *et al.*, 1991). Damage to the right hemisphere can produce either no immunological change, or even enhance activity of certain immune parameters (Goldstein *et al.,* 2002; Neveu, 1988, 1991, 1992). Furthermore, a study using rats with implanted electrical cortical stimulation revealed that stimulation of the left temporo-parieto-occipital cortex temporarily increased production of thymic $CD4^+$ and $CD8^+$ lymphocytes, while stimulation of the right hemisphere decreased their levels (Moshel *et al.*, 2005).

Geschwind and Behan (1982) developed a theory based on the association of prenatal testosterone exposure and termed this "anomalous dominance" (i.e. right sided "abnormal" language lateralisation). Observing higher incidences of left-handedness amongst individuals with developmental and immune disorders, they hypothesised that this anomalous dominance was a contributor for variation in susceptibility to illnesses (Geschwind & Behan, 1982; Morfit & Weekes, 2001). This theory was supported through large population surveys of left handed individuals, and studies of sinistrality in patients with migraine and immune disorders (Geschwind & Behan, 1982). Whilst vital to the investigation of effects of HL on immune function, this research had conceptual and methodological flaws, most notably the definition of "anomalous dominance". The latter cannot be determined by handedness alone, and is too broad a concept in itself to be used as a definition of cerebral dominance pattern. Handedness is only one of many activities and behaviours that are lateralised and cannot comprise a total asymmetry index (McManus & Bryden, 1991). Furthermore, handedness is very poorly correlated with HL, but rather may be indicative of language lateralisation only, as language lateralisation in sinistrals is heterogeneous, whereas with dextrals it is almost exclusively in the left hemisphere (Gruzelier *et al.*, 1996; Jung *et al.*, 2003; Knecht *et al.*, 2000; Toga & Thompson, 2003). Moreover, a study using mice showed that handedness effects on immunity were abolished with

left, but not right, cortical ablation (Neveu *et al.*, 1991), suggesting the involvement of more complex brain organisation factors in the HL-immunity relationships. While handedness is related to hemispheric *specialisation* (e.g., left hemisphere specialising in certain linguistic abilities), people may differ in their relative levels of hemispheric *activation* (Davidson *et al.,* 1999), which we refer to here as hemispheric lateralisation (HL) or cerebral activation asymmetry. Below we provide a more comprehensive definition of HL.

The purpose of this review

Using electroencephalograms (EEG) and neuroimaging techniques, as well as neuropsychological tests assessing specific brain functions, we can test more precise relationships between the CNS and immune systems, with particular emphasis on HL and immunity. To date, there have been no systematic reviews of the research on the relationship between HL and immunity in humans, nor do we know the magnitude of such a relationship. This could be, at least in part, due to the fact that laterality itself is difficult to define – with the term being mostly applied to cognitive attributes. The present review aims to synthesize the currently available research, assess its methodological quality and effect sizes, delineate study limitations, and interpret the findings with a view to future advancements in the research area and its clinical implications.

Definition of Hemispheric Lateralisation in Neuroimmunology

For the purposes of this review, the definition of HL for neuroimmunology should be separated from that used in cognitive neuroscience. The latter uses HL in the context of *hemispheric specialisation,* whereas the broader concept of *hemispheric activation* is the basis for this area of neuroimmune theory. It has been suggested that hemispheric activation can actually influence hemispheric specialisation (Davidson *et al.*, 1999; Davidson & Hugdahl, 1996; Kang *et al.*¸1991), which means that focusing on the cognitive terms of specialisation alone may not reflect the true antecedents of the immunological influence. Moreover, activation can be independent of hemispheric specialisation (Davidson & Hugdahl, 1996).

METHOD

Literature Search

A computer-based search was conducted for the present review from the following databases; OVID, CINAHL, EBSCOhost EJS, Ingenta Journals, NCBI PubMed, Science Direct, Highwire Press, Scopus, Springer Link, Taylor & Francis Journals and Wiley Interscience. The key search terms of "lateralisation" and "lateralization" were combined with the Boolean operator OR; in conjunction with AND for the secondary terms of "immune function", "T cells", "natural killer cells", "immunity", "cytokines", "lymphocytes". Another strategy of using as keywords "cerebral asymmetry", "stroke", "epilepsy", "traumatic brain injury", and "cerebral lesion" were combined with the Boolean operator OR, in conjunction with AND for the secondary terms listed previously. The time period selected was to encompass the years from 1982 (the year of the Geschwind & Behan study) to 2010. Only full text articles were identified for inclusion into the review. There were no systematic reviews or meta-analyses currently available on this topic in humans. All identified articles were scrutinized for other relevant articles in their reference lists.

Selection Criteria

Selection of studies was based upon their relevance to humans, and their relevance to relationships between lateralisation and aspects of immune functions, using either a cross-sectional or experimental design. Studies that did not primarily assess this relationship were also considered if their data examined this as a secondary analysis. This systematic review was limited to papers published in English or French. Studies that were excluded include those not employing functional or activational assessments of laterality (such as handedness), and those using clinical conditions that resulted in more complications than just lateralisation (e.g. cerebral palsy). Studies that did not employ direct immunological measures as an outcome measure (i.e. self-report health surveys) were excluded.

Quality Assessment

In the absence of a standardised checklist for such heterogeneous data, a quality assessment scale was developed from quality assessment frames such as can be found in Mols *et al*. (2005) and Borghouts *et al.* (1998). The quality of each of the articles

was assessed using a five-item checklist, with scores ranging from 0 to 18, with relevant predefined criteria (see figure 1). The categories viewed essential to ensuring a good standard of methodological quality (internal validity) concerned control over third variables and the statistical treatment. Those third variables that can affect the relationship between the brain and the immune system were demographic (e.g., age), psychological (e.g., chronic stress), physiological or neurological (e.g., inflammatory diseases), and an extensive list of these variables was established. Another essential criterion of quality assessment was the study conclusions: whether correctly derived from the study design and results. One investigator (RS) assessed all articles and another (YG) assessed five of the articles for inter-rater reliability. The quality criteria are presented in Figure four.

Extracted information

Summary of extracted details from the studies included: research team, year of publication, sample details, types of HL and immunological tests, research design and results.

Effect Size Assessment

Effect size calculations were undertaken only on the main tests of the relationship between HL and immune functions. These were calculated utilising the reported information relevant to the statistical tests used for each main relationship or effect studied. Mean effect sizes across study type (cross-sectional, semi-experimental) and across all studies were calculated to yield a single scale for comparison across studies. This procedure was again employed to assess across groups of activation and functional analysis.

RESULTS

Study Characteristics

After all exclusion criteria were applied to the identified literature, 11 articles were eligible for inclusion in the present review. Eight studies used clinical samples (e.g. stroke, epilepsy patients) and three used healthy samples. Eight of the studies used quasi-experimental or experimental methodologies; and three used cross-sectional or prospective methodology. A summary of the reviewed studies can be found in Table two.

1) Design (scoring 0-2)

0= cross-sectional
1= quasi-experimental (e.g. using clinical groups as "experimental" conditions)
2= experimental
An additional point is offered if the results are compared between two or more groups

2) Sample (scoring 0-2)

0= small (<40)
1= moderate (40-79)
2= large (80+)

3) Third Variables (scoring 0-5)

0= none considered
1= a total of 1 or 2 (from one or more groups) (see below)
2= a total of 2-4 (from one group)
3= a total of 2-4 (from two or more groups)
4= a total of 4+ (from one group)
5= a total of 4+ (from two or more groups)

Groups:

- Psychological – Anxiety/depression, current mood, life events, family, sexuality, employment status and type, IQ.
- Physiological – Comorbid illness, medications, activity/lifestyle, prior dependence of drugs/alcohol, current use of nicotine and caffeine.
- Neurological – ANS interactions, handedness, language lateralisation, previous TBI or neurological disorder.
- Background – SES, age, education, gender.

4) Statistics (scoring 0-4)

0= 0/4 suitability criteria
1= 1/4
2= 2/4
3= 3/4
4= 4/4

Criteria:

- Appropriate choice (parametric assumptions, degrees of freedom, prospective power analyses, bivariate vs multivariate, data transformations)
- Control for third variables (baseline adjustments, missing data, sphericity adjustments etc.)
- Setting of *p* value to .01
- Post-hoc analyses (further stat testing, power analyses, mediating/moderating effects, prospective assessment for further testing if applicable)

5) Conclusions (scoring 0-4)

0= 0/4 suitability criteria
1= 1/4
2= 2/4
3= 3/4
4=4/4
Criteria:

- Conclusion validity
- Limitations
- Contextual evaluation
- Indications for future research

Figure 4. Quality assessment criteria for the review of studies examining hemispheric lateralisation and immunity

Table 2. Overview of studies on the relationship between hemispheric lateralisation and immune function

Paper	Sample	Brain Measures	Immune Measures	Design	Results	Quality Score 0-17
Kang *et al*. 1991	20 (11 extreme stable right activation; 100% f, 17-20 years) Right handed in top or bottom 25th percentile of activation asymmetry.	EEG Also state-trait anxiety scale, BDI and Derogatis Stress Profile. (Activity)	NK, lymphocyte proliferation (ConA, PHA, PW), T cell subset, plasma immunoglobulins and plasma cortisol. Also self-made questionnaire detailing frequency of common illnesses in preceding 2 and 12 months, family history of autoimmune diseases.	Cross-sectional	Lower levels of NKCA and IgM found in s' with frontal right activation as opposed to left. – not extended to T cell subset profile or lymphocyte proliferative response to ConA or PW. Proliferation to PHA was in the same direction as NKCA. Magnitude of difference in NKCA across both L and R groups was similar to the magnitude of difference in NKCA in stressful events. Immune patterns not accounted for by health survey, plasma cortisol levels, anxiety or depression.	9
Tarkowski *et al*. 1995	80 (51.25% f) stroke patients.	Hachinski method of assessing minor, major or progressive stroke. Lateralisation of stroke assessed by physical exam and CT scan. (Activity)	Tuberculin for skin reaction. Histamine injection for T-cell mediated immune response. Axon reflex vasodilation. Stimulation of PBMCs to PPD, PHA and ConA to yield production of IFN-γ.	Quasi-experimental	Lateralisation of DTH response dependent on stroke clinical categorisation, motor function and side of lesion. Those with lesion on the right side had significantly larger DTH responses than those with left lesions which is independent of clinical categorisation of stroke.	7

Paper	Sample	Brain Measures	Immune Measures	Design	Results	Quality Score 0-17
Gruzelier *et al.* 1996	36 (27 with longitudinal assessments) asymptomatic HIV-1 infected patients, 100% male, 11.1% non-dextral.	EEG, WRMT, controlled verbal fluency, semantic processing test, finger tapping, grooved peg-board test, HADS, POMS. (Activity & Function)	CD4 and CD8 taken at study onset and at 6 month intervals for 30 months.	Cross-sectional	Superior left hemisphere functioning associated with higher CD4 count at baseline and through to study end. No relationship found between EEG activation asymmetry and CD4 count. Superior right functioning in WRMT indicated increased immune suppression (CD8). Higher POMS at onset predictive of poorer immune outcomes throughout study.	7
Tarkowski *et al.* 1998	117 in total split into 3 groups: 1) 44 early stroke patients (47.8% f, 23-81yrs) 2) 24 early stroke with retest (45.8% f, 23-76yrs) 3) 49 chronic stroke (40.8% f, 23-88yrs).	Hachinski method of assessing minor, major or progressive stroke. Lateralisation of stroke assessed by physical exam and CT scan. (Activity)	Tuberculin for skin reaction. Histamine injection for T-cell mediated immune response. Axon reflex vasodilation. Stimulation of PBMCs to PPD, PHA and ConA to yield production of IFN-γ.	Quasi-experimental	Replicated former findings that DTH responses stronger in patients with right localised ischemic trauma, but also that those with right side trauma showed significantly different effects with time. The later challenges created greater immune effects on the paretic side later in the post-stroke period compared to those in the earlier challenges.	6

Paper	Sample	Brain Measures	Immune Measures	Design	Results	Quality Score 0-17
Davidson *et al.* 1999	24 healthy s', 37.5% female, 17-21 years	EEG Separately validated emotion eliciting film clips. (Activity)	NKCA approximately 4 weeks after EEG data taken.	Experimental	Superior anterior left sided activation predicted higher NKCA (mid-frontal, lateral frontal and anterior temporal regions). Differences in posterior activation unrelated to NKCA measures. Lateral prefrontal activation asymmetry accounted for 21% of the variance in NKCA at final exam time, even when accounted for by baseline measures. S' with greater left frontal activation showed higher levels of NKCA after the positive film clip, this was exceeded by final exam effects which were three times greater.	10
Meador *et al.* 1999	11 surgical epilepsy patients: 20-48 years; 18.1% f; 8 R handed with L language dominance, 1 ambidextrous with L language dominance, 1 R handed with bilateral language representation (excluded from statistical analysis), 1 L handed with R language dominance (R temporal resection). 5 right, 5 left temporal lobectomies, 1 left frontal lobe resection.	Clinical diagnosis of epilepsy location and language lateralisation (language dominant resection = DOM; language non-dominant resection = NDOM). (Function)	Complete Blood Count (CBC): Total White Blood Cells (WBCs) Total $CD3^+$ $CD3^+4^+$ $CD3^+8^+$ $CD8^+$ Total lymphocytes Blood taken the day before surgery and a mean of 6 days after surgery.	Quasi-experimental	No significant main effects found aside from an elevation in WBCs in pre- to postoperative states across all patients. Interactions for DOM and NDOM groups for pre-/postop states were found for absolute lymphocyte, $CD3^+4^+$, $CD3^+8^+$, and total $CD8^+$. Follow-up contrast *t* tests performed showed significant results for increase in WBCs and decline in $CD8^+$ for the DOM group. The NDOM group showed significant increase in $CD3^+4^+$. All other *t* tests for the DOM group were non-significant, but were in the trend of the non-significant main effects; absolute lymphocyte decline, $CD3^+4^+$ decline, $CD3^+8^+$ decline, and total $CD8^+$ decline. The pre- and postoperative changes were in opposite directions for NDOM (increase) and DOM (decline) groups, aside for WBCs which were universal increase.	8

Paper	Sample	Brain Measures	Immune Measures	Design	Results	Quality Score 0-17
Ivashkova *et al.* 2002	Clinical group – 38 subacute stage stroke patients receiving TMS, 44-64yrs, 52.6% right hemisphere location. Control group – 30 healthy Reference group – 35 subacute stage stroke patients not receiving TMS, 54.3% right hemisphere.	None mentioned aside from stroke assessment. (Activity)	CD3+, CD4+, CD8+ and CD22+ BTR of human leukocytes (ConA, PHA, PW) Lymphocyte suppressor activity Phagocytic activity of neutrophils (NTR) Leukocyte adhesion suppression.	Quasi-experimental	The type and degree of immune alterations was dependent on the lateral location of the lesion. Right hemispheric stroke resulted in $CD3^+$ and $CD8^+$ decrease, and $CD4^+/CD8^+$ ratio increase and disturbances in lymphocyte proliferation activity compared to healthy controls. Left hemispheric stroke resulted in $CD3^+$, $CD4^+$ and $CD8^+$ decrease as well as disturbances to lymphocyte proliferative activity in comparison to healthy controls. TMS of sensory and motor regions of the cortex of right hemispheric stroke patients caused normalisation of immune values. Change less pronounced after TMS for left hemispheric patients.	8
Clow *et al.* 2003	16 healthy participants, 37.5% f. Two males and one female studied twice.	TMS to both hemispheres separately. (Activity)	Salivary S-IgA and salivary volume.	Experimental	TMS to the left hemisphere causes upregulation of S-IgA. TMS to the right causes reduction in saliva volume. S-IgA rises after TMS on both sides but with salivary volume falling there is a significant increase after left TMS rather than right.	8
Dziedzic *et al.* 2003	Reference Group – 26 right handed stroke patients; 11 right lesion (45.5% f; mean age 63.7); 15 left lesion (46.7% f; mean age 60.6). Control group – 16 healthy s' (43.8% f, mean age 62.3).	Stroke location and size determined by CT scans. (Activity)	Serum IL-10 and IL-6.	Cross-sectional	Both left and right stroke patients had significantly higher levels of IL-10 and IL-6 than healthy controls. IL-10 was higher in left stroke patients and there was no difference in IL-6 in either patient group.	9

Paper	Sample	Brain Measures	Immune Measures	Design	Results	Quality Score 0-17
Meador *et al.* 2004	22 surgical epilepsy patients; 19-61 years, 45.5% f, 81.8% left language dominant (others mixed), half right resection half left. Healthy controls.	Intracarotid amobarbital test for language dominance. POMS and daily stress inventory taken. (Activity)	CBC Lymphocyte subset analyses Mitogen and microbial responses Histamine skin testing Cortisol	Quasi-experimental	Lymphocytes, total T cells, cytotoxic T cells and T helper cells decreased with left and increased with right resections; these effects were unaltered when mood and cortisol accounted for. No differences in mitogen and microbial responses. Greater right arm histamine wheal responses found in left-brain dysfunction as compared to right and control. Histamine flare responses decreased after left resection and increased in right resection patients as compared to right sided patients and controls.The four patients with atypical language lateralisation had left lesions and surgery, cellular and skin responses differed from left resection patients with normal language lateralisation.	7
Koch *et al.* 2006	56 acute stroke patients (11 TIA, 17 Lacunar, 20 atherothrombotic, 8 cardio-embolic). Mean age 58.9, 46.4% female, 31 left localisation of stroke, 25 right localisation.	MRI/CT for localisation of stroke trauma. (Activity)	C-Reactive Protein (CRP) and WBC (retrospective collection from medical notes)	Quasi-experimental	Left hemispheric stroke resulted in increased variability in CRP and WBC, and higher absolute values of CRP and WBC. Correlation between CRP and WBC only observable in left-sided stroke patients.	8

Quality Assessment

To assess inter-rater reliability of quality assessment, scores were correlated using simple bivariate correlation with SPSS. The correlation between the two evaluators for the quality assessment of the five articles dually rated was 0.98. The quality assessment figures are presented in Table two. The mean quality assessment mark was under 50% (7.909, SD= 1.136) of the total possible score, 17. The general low level of methodological quality of the studies was mainly due to their lack of control over third variables, limited sample sizes and questionable inferential validity.

Effect Sizes

Effect sizes (*r*) were calculated for the main effects of nine of the reviewed studies for which relevant data were available for these calculations. A mean effect size for the HL-immunity relationship was calculated per study, across its various tests. The effect sizes were interpreted using Cohen's (1988) criteria for small ($r > 0.1$), medium ($r > 0.3$) and large ($r > 0.5$) effects. Using these criteria, five of the nine studies (67%) had large mean effect sizes for their main effects; two showed a medium effect size; and two a small effect (Table 2). The mean effect size (*r*) of the HL-immunity relationship for the main effects of the assessed studies was 0.532, which is designated as a large effect size. Of the three cross-sectional studies reviewed, two provided sufficient data to process effect size analyses; the mean effect size for these two studies was 0.533. Of the eight remaining studies that employed either experimental or quasi-experimental designs, seven provided sufficient numerical data to conduct effect size calculations. The mean effect size for this group of studies was 0.537. This effect size refers to the overall differential effect of lateralisation on immunity. A summary of the effect sizes obtained for the assessed studies can be found in Table three.

Each study was also classified as to whether HL was measured according to activity or function. Studies including patients after stroke or epilepsy, using EEG or brain stimulation, were classified into the activity HL group. Studies using neuropsychological tests or linguistic dominance were classified into the functional HL group. It is important to note that this classification is not perfect since lesions due to stroke or epilepsy could be construed as both reflecting changes in activity and function. Nevertheless, we related them to the activity group since changes following stroke, for example, are marked first by reductions in neuronal activity (Jiang *et al.*,

2010). We then classified studies into activity versus functional measures of HL, since the studies utilized either or both types. Functional measures (2 studies alone) yielded an effect size of 0.63 while activity measures (8 studies) yielded an effect size of 0.515.

Quasi-Experimental and Experimental Data

The studies in this group involved either direct manipulation of variables (Davidson *et al.*, 1999; Clow *et al.*, 2003) or used patient groups that demonstrated atypical lateralisation (Koch *et al.*, 2006; Ivashkova *et al.*, 2002; Meador *et al.*, 1999; 2004; Tarkowski, *et al.*, 1995; 1998). The articles are presented chronologically, according to the year of publication.

Tarkowski et al., 1995

Using patients of minor, major and progressive stroke, Tarkowski *et al.* (1995) assessed tuberculin skin reaction, histamine initiated T-cell response, and pokeweed (PW), phytohemagglutanin (PHA) and Concanavalin-A (Con-A) initiated lymphocyte distribution in those with left or right localised brain trauma. Lateralisation of skin response was relative to the clinical categorisation of the stroke, residual motor function and the lateral localisation of the lesion. Patients with left localised lesions (n=24) displayed smaller delayed type hypersensitivity (DTH) responses than those with right lesions (n=26), but those with right-sided lesions also demonstrated lateralised peripheral reactions (i.e. greater responses on ipsilateral side than contralateral side). The ability to find a HL-immune relationship in such a heterogeneous sample, compared to the more homogenous samples described below, supports the generalised effect of HL on immune function in humans. However, very little control over third variables was undertaken (e.g., age, hypertension, medications), which is particularly important in patients with cerebral ischemia. Stroke may be indicative of other illnesses that may affect the communication between the brain and immune system, and such illnesses could in themselves be indicative of atypical brain to immune communication (e.g. hypertension, diabetes), possibly affecting vagal immune-modulation. No control over psychological or cognitive variables relevant to stroke, that may be related with either HL or immune

function (e.g., executive functions, depression), was conducted either, limiting the inferential validity of the findings. It should also be noted that allergic responses in the skin can also be influenced by handedness (Bryden *et al.*, 2005); which could have implications for the interpretation of the present findings.
Classification: Activity

Tarkowski et al., 1998

In a follow-up study, Tarkowski *et al.* (1995) employed the same methodology, but divided the clinical sample of stroke patients into three groups: early stroke, early stroke with retest of parameters in the subacute phase, and chronic stroke. This study replicated the former findings, but also showed that the early stroke group with localised right trauma exhibited larger DTH responses to immune challenges. This was observed in both the paretic and contralateral sides in comparison to those with left stroke. In the chronic phase of stroke, those with right localised ischemia showed a greater response than those with left stroke at the ipsilateral side. This study also considered a substantial number of third variables, although there were no controls for levels of depression, anxiety or general mood, which are related to immune function (Kemeny & Schedlowski, 2007), and are clinically relevant in such patient samples.
Classification: Activity

Davidson et al. 1999

In a sample of healthy students Davidson *et al.* (1999) used emotionally evocative film clips to manipulate natural killer cell activity (NKCA) in left and right lateralised participants, as determined by electroencephalography (EEG). NKCA was assessed at an anxiety-neutral time (mid-semester) and at a high anxiety time (exam period). Participants with left frontal-anterior-temporal lateralization had greater NKCA in response to the positive film clips at both times than those with right lateralization. This study thoroughly considered third variables including handedness, caffeine, nicotine and alcohol consumption. However, there was no control for the use of prescription medications, or other illnesses, that may interfere with natural brain or immune system functioning, and this study had a relatively small sample size (n= 24) in comparison to the mean of all studies (n= 47.1). Nevertheless, using a naturalistic

stressor (exam stress) and finding higher NKCA in left-HL participants in two contexts suggests that this relationship may be generalisable to various contexts.
Classification: Activity

Meador et al., 1999

Including patients waiting for epilepsy resection surgery, Meador *et al.* (1999) examined the differences in those receiving surgery at language dominant (DOM, left) and non-dominant (NDOM, right) hemispheres, in relation to several immunological outcomes. Resection surgery is employed with intractable epilepsy, whereby an area of the affected lobe is excised in order to diminish the epileptogenic activity. Increases in white blood cells (WBC) and decreases in CD8 post-surgery for the DOM group, and increases in CD4 post-surgery for the NDOM group were observed. Overall, aside from total WBC counts, the immune parameters decreased post-surgery for the DOM group and increased for the NDOM group, although many of the findings did not attain statistical significance. Of the main effects, only the total pre-operative versus post-operative group analysis of WBC attained significance, the DOM versus NDOM main effect did not reach significance. A number of *post-hoc* tests were employed, although it is not clear whether statistical adjustments were made for these multiple comparisons. However, the overall observed trend was in line with right hemisphere functioning being indicative of poorer immune functions. The sample size for this study was relatively small (N=11), and it was heterogeneous due to its clinical nature. There was relatively little third variable control (e.g., comorbid illnesses). Whilst the use of the DOM/NDOM classification is useful to partly understand the HL of the patients; looking at resections in these categories as opposed to absolute left/right hemispheres, is conceptually problematic as it defines laterality in relation to function or specialisation, rather than to activity and side. It is known for example that not all individuals have a left side language dominance.
Classification: function & activity

Table 3. Table of effect sizes for the main effects of the reviewed research concerning hemispheric lateralisation and immune function

Paper	Effect	Effect Size (*r*)	Mean *r* for the Study
Kang *et al.*, 1991	Natural Killer Cell Activity (NKCA) – Left vs Right – overall	0.678	
	Lytic Units at 30%	0.559	
	IgM – Left vs Right	0.527	0.588
Tarkowski *et al.*, 1995	Lateralisation of stroke-induced lesion & DTH – Left vs Right	0.280	0.280
Gruzelier *et al.*, 1996	Word fluency (Left) and CD4	0.610	
	Semantic processing errors (Left) and CD4	0.480	
	Finger tapping asymmetry (dominant hand) and CD4	0.540	
	Memory for faces (Right) and CD8	0.530	0.540
Tarkowski *et al.*, 1998	Early stroke & DTH – Left vs Right	0.862	
	Right subcortical lesions & DTH – Paretic vs Contralateral	0.675	0.769
Davidson *et al.*, 1999	Baseline frontal asymmetry & baseline NKCA (11:1 ratio)	0.460	
	Baseline frontal asymmetry & baseline NKCA (33:1 ratio)	0.510	
	Baseline lateral frontal activation & baseline NKCA (11:1 ratio)	0.410	
	Baseline anterior temporal activation & baseline NKCA (11:1 ratio)	0.480	
	Baseline anterior asymmetry & NK reactivity to emotional film clips	0.440	0.460
Meador *et al.*, 1999	DOM vs NDOM group – Pre- to Post-op – Absolute Lymphocytes	0.647	
	DOM vs NDOM group – Pre- to Post-op – Total $CD3^+$	0.675	
	DOM vs NDOM group – Pre- to Post-op – $CD3^+CD4^+$	0.666	
	DOM vs NDOM group – Pre- to Post-op – $CD3^+CD8^+$	0.675	
	DOM vs NDOM group – Pre- to Post-op – $CD8^+$	0.678	0.669
Ivashkova *et al.*, 2002*	Right vs Left hemispheric stroke pre-TMS – cellular immunity (mean value)	0.794	
	Right vs Left hemispheric stroke pre-TMS – neutrophil activity (mean value)	0.938	0.866
Clow *et al.*, 2003		Not Available	

Dziedzic *et al.*, 2003		Not Available	
Meador *et al.*, 2004	Lymphocytes – Left vs Right across Pre/Post operative	0.535	
	Total T cells – Left vs Right across Pre/Post operative	0.587	
	Helper T cells – Left vs Right across Pre/Post operative	0.587	
	$CD3^{+}8^{+}$ – Left vs Right across Pre/Post operative	0.494	0.551
Koch *et al.*, 2006	C-Reactive Protein level – Left vs Right	0.125	
	White Blood Cell level – Left vs Right	0.196	0.161

Effect Size (*r*) designations (Cohen, 1988)
Small = 0.1
Medium = 0.3
Large = 0.5

* This study involved multiple interactions between 11 immune parameters (5 cellular immunity, 6 neutrophil activity) and four interactions (Left vs Right; Pre TMS vs Post TMS; Control vs Left and Right – Pre TMS; Right Pre & Post vs Left Pre & Post). To avoid reporting all 77 calculated effect sizes, the mean effect size of all of those in that section (T cell or lymphocyte proliferation) was used to illustrate the main lateralisation finding of the study.

Ivashkova et al., 2002

Ivashkova *et al.* (2002) used three groups of participants; a group of subacute stage stroke patients who received transcranial-electromagnetic stimulation (TeMS) therapy; a reference group of subacute staged stroke patients who did not receive TeMS, and a control group of healthy participants also receiving TeMS. A variety of immunological parameters were assessed, along with lymphocyte proliferation to Con-A, PW and PHA before and after TeMS, and proliferation changes were compared to the values observed in the control and reference groups. Right hemispheric stroke was shown to be related to T-cell deficit and disruption of lymphocyte proliferation, whereas left hemispheric stroke was associated with decrease in lymphocyte proliferation only. TeMS resulted in the normalisation of immune values in the group of clinical subjects with right localised lesions. The main finding of the study was that lateral localisation of the lesion was directly involved in the type and degree of observed immune alteration. The main limitation of this study is the disparity between study groups, making comparison between these groups more circumspect. The control group (N=30) was compared against two clinical groups (total N=73); and the TeMS and non-TeMS groups were equally disproportionate (N= 68, 35 respectively). Methodologically, the study is also limited by the use of multiple statistical tests, risking a type 1 error, without employing an appropriate correction. They report performing an extremely large number of t-tests (77), and if the standard Bonferoni correction for multiple comparisons, dividing the p critical value (0.05) by the number of t-tests (77), were applied it is unlikely that many (if any) of the results would survive. However, the use of a control group and a reference group was a methodological strength. Finally, the duration, frequency and precise location of the TeMS were not reported, which may impact the inferential validity of the findings as these factors affect immunity (Davidson et al., 1999). Analysis of confounder control either methodologically or statistically is difficult to infer, as there were no exclusion criteria or extraneous variable controls mentioned.

Classification: Activity

Clow et al. 2003

Healthy participants were selected for this study, which involved using repetitive transcranial magnetic stimulation (rTMS) to both hemispheres (on separate occasions) over the temporo-parieto-occipital (TPO) cortex, and assessing salivary Immunoglobulin A (S-IgA) changes before and after stimulation. The authors reported that initially rTMS to either hemisphere resulted in an increase in levels of S-IgA. However after accounting for saliva volume, revealing the concentration of S-IgA, the results were further elucidated: left hemispheric rTMS resulted in an increase in S-IgA whereas right resulted in decreases in S-IgA. Nonetheless, with a sample size of just 16 participants, with 3 participants being tested twice meant this preliminary study was relatively small in comparison to the rest under review. Furthermore, it is unclear as to how the data from those participants who were retested was dealt with, which makes validity difficult to assess. The only inclusion criteria mentioned were that the participants were healthy and right handed, with no mention of exclusion factors or control for third variables. Finally, there was also no "sham" rTMS condition, which could affect the results (Toschi *et al.*, 2009), and the inferences concerning the effects of rTMS and HL on immunity.

Classification: Activity

Meador et al., 2004

This research group again used surgical epilepsy patients to examine post-surgical changes in immune parameters. This study also used analyses of the same variables in a healthy control sample to control for variability in these measures. Using lymphocyte counts, responses to mitogen and microbes, and histamine skin testing, they also examined the effects of mood (Profile of Mood States; POMS) in the relationship between hemispheric surgery location and immune alteration. Left resection patients showed decreases in total lymphocytes, T cells, CD8 and CD4 after resection surgery, while the opposite was observed for the right resection patients. These effects remained stable when POMS was included in the statistical testing, suggesting that mood is not a moderator in the relationship. Histamine skin responses

showed that left resectioned patients displayed greater right arm wheal responses compared to the right resection patients and control group. Flare responses were reported to decrease after left resection, and increase after right resection. In comparison to the previous Meador *et al.* (1999) study, this research included twice as many participants (n=22), but is still below the mean for all of the studies reviewed. The change in methodology from examining differences between DOM and NDOM groups, to differences between left and right resection groups makes the findings more directly relevant to HL and more coherent. The use of cellular proliferation tests and histamine reaction in a control group to account for non-systematic variability in the clinical group, and the examination of psychological variables (mood) demonstrates carefully considered confounders and processes, although no exclusion criteria were detailed. The finding of left resection leading to both reduced cellular (Th1) immunity (decreased T-lymphocytes) and increased allergic responses (greater histamine reaction) is indicative of the left hemisphere being implicated in the modulation of immunity and possibly in certain immune-related illnesses. This association, however, may well be a conflicting one, as Th1 immunity involves the expression of pro-inflammatory cytokines, which would be decreased in this sub-sample, but allergy requires an increase in Th1 immunological response (Webster *et al.*, 2002). Further research is required to expand upon the different immunological consequences of left HL. Nevertheless, concerning only the lymphocyte data, these results are in line with a differential (and inverse) immunological function of HL.

Classification: Activity

Koch et al., 2006

In the more recent of the reviewed studies, Koch *et al.* (2006) also used stroke patients to examine the effects of stroke lateralisation (as verified by magnetic resonance imaging or CT) on C-reactive protein (CRP) and WBC. The authors reported that left hemispheric stroke resulted in an increased variability in both CRP and WBC, and that correlations between these two parameters were only observable in the left-localised stroke patients, interpreted by the investigators as suggesting a deficit in immune control after left-sided ischemia. This study limited its examination to the immune parameters of WBC and CRP, which limits the comparison to similar

studies under review here (e.g. Meador *et al.*, 2004) due to the non-specificity of such markers. Furthermore, the immune measures were only conducted in the first 24 hours since stroke onset. Stroke in general, regardless of laterality, has been suggested to cause alterations to lymphocytes, granulocytes and leukocytes, particularly within the first 24 hours of onset (Miller *et al.*, 1991; Vogelgesang *et al.*, 2008), which would necessitate testing HL-immune relationships at periods beyond this phase, as these changes could be partly stroke-related rather than laterality-related. This is in stark contrast to the methods employed by the Tarkowski work groups (Tarkowski *et al.*, 1995; 1998); who assessed immune alterations longitudinally across the course of stroke clinical staging. In addition, no theoretical rationale for examining the relationship between CRP and WBC was provided. Does a greater variability in these two immune measures exist among left-hemisphere people, or does the left hemisphere in general regulate one or both parameters, possibly influencing the other one? Finally, what does lack of a correlation between CRP and WBC mean biologically? Thus, beyond methodological limitations, the interpretation and meaning of the observed results remain problematic.

Classification: Activity

Cross-Sectional or Prospective Data

Three studies involved using healthy (Kang *et al.*, 1991) and clinical (Dziedzic *et al.*, 2003; Gruzelier *et al.*, 1996) participants; and they are presented in chronological order.

Kang et al., 1991

Using EEG measures, the researchers selected a group of healthy participants who displayed “extreme stable activation”; designated as those in the upper and lower quartile of prefrontal activation asymmetry. The researchers examined NKCA, lymphocyte proliferation (to Con-A, PHA and PW) and other immune parameters, whilst also obtaining self-report data concerning frequency of common illnesses in the past 12 months and family history of autoimmune diseases, as well as administering some psychometric scales (anxiety, depression and stress). They reported that higher right frontal activation (as opposed to higher left) resulted in lower levels of NKCA

and Immunoglobulin-M, as well as lower lymphocyte proliferation in response to PHA. The immune effects observed could not be accounted for by the health survey, plasma cortisol levels or the psychometric scales. The use of subjective self-report data concerning health may raise questions concerning validity of immune related illnesses. Nevertheless, the methodology was thorough including details about viral, fungal and respiratory infections as well as allergies and dermatological status. Control for confounding variables, the selection of participants in the top and bottom quartile for HL, taking details of drug use, including right-handed participants only, and conducting the immunological assessments at an anxiety-neutral time, are all evidence of relatively strict methodology. However, the small sample of female participants alone also has an impact on the generalisability of the findings, and leaving immunological assessment possibly subject to hormonal influences which were not fully tested or controlled for (Butts & Sternberg, 2008; Kovats & Carreras, 2008; Taub, 2008). The observed lack of cortisol effects could mean that HL-immunity relationships are dependent on other neuro-endocerine-immune pathways, unrelated to the HPA axis or only unrelated to cortisol. We shall discus this important issue below.

Classification: Activity

Gruzelier et al., 1996

This study included asymptomatic HIV^+ patients, and measured their immune outcomes (CD4, CD8) at a follow-up of 36 months in relation to baseline EEG recordings of cerebral laterality and performance on neuropsychological tests assessing right/left brain functions. The experimenters found that greater left hemisphere functioning predicted higher CD4 both at baseline and at follow-up, and that greater right functioning predicted greater immune suppression (CD8). The sample for this study was small (N=27) and was restricted to men of bisexual or homosexual orientation. Today, the largest proportion of HIV transmission across the world occurs in heterosexual activity (Grant & De Cock, 2001; Hansasuta & Rowland-Jones, 2001). The pathogenesis of HIV may depend on biological parameters and socioculturally influenced health behaviours of individuals (i.e., comorbid illness, clinic attendance, heavy drug or alcohol use) which was not and cannot be accounted for in such a restricted sample, nor generalised to a wider

population (Derdeyn & Silvestri, 2005; Lama & Planelles, 2007; Gifford *et al.*, 2002). Most importantly, the investigators did not statistically control for effects of baseline immune parameters and other confounders (e.g., education, mode of infection, other illnesses, medications) that may affect the autonomic, nerve or immune systems (Cole *et al.*, 2003). All these limitations question the validity of their inferences. Nevertheless, reviewed here, the Gruzelier *et al.* (1996) study shows a prospective relationship between two different measures of HL, function and activity, with immunity in the context of an immune-related illness. However, to ensure these findings are valid, future studies must replicate it and address its many limitations.

Classification – Activity & Function

Dziedzic et al., 2003

This study used stroke patients to examine the relationship between stroke location and interleukin (IL)-10, and IL-6. Stroke location and size were assessed using CT scans. An age- and gender-matched control group was used to compare general immunological parameters, but were not assessed for any form of lateralisation and so were not included in the analysis of the main effect. The stroke patients showed higher IL-10 and IL-6 levels than the control group. Within the stroke patient group, those with left localised stroke showed higher levels of IL-10, but there was no difference in IL-6. This study exhibited a good level of confounder control, with psychological, physiological and neurological factors all being considered, as well as including a healthy control group. However, as with Koch *et al.* (2006), the immune measures were taken at around 24 hours after hospital admission, which makes the reliability of the finding of abnormal IL-10 questionable, as this may not be due to laterality *per se*, but possibly also due to the stroke itself. Whilst IL-6 and IL-10 reflect Th1 and Th2 immunity, respectively, a wider panel of immunological assessment including cytokines which clearly reflect cellular immunity activity (e.g., Interferon-gamma) would have been useful. Nevertheless, this is one of the only studies examining the relation between HL and cytokines, and results suggest that left HL is related to lower anti-inflammatory activity (IL-10). More studies need to replicate and extend this important issue.

Classification - Activity

DISCUSSION

General conclusions

This systematic review summarises the results of 11 research articles investigating the relationship between hemispheric lateralisation (HL) and immune function. All of the reviewed studies show a relationship between HL and immune function. Three of the 11 (27.3%) studies describe a relationship between poorer left versus right hemisphere function and decreased immunity in at least one immune parameter (Dziedzic *et al.*, 2003; Kang *et al.*, 1991; Koch *et al.*, 2006). Three of the 11 (27.3%) studies describe a relationship between poorer right versus left hemisphere function and increased immunity in at least one parameter (Davidson *et al.*, 1999; Tarkowski *et al.*, 1995, 1998), in line with the finding of the first three studies. Five of the 11 (45.4%) studies describe both relationships of HL and immunity (Clow *et al.*, 2003; Gruzelier *et al.*, 1996; Ivashkova *et al.*, 2002; Meador *et al.*, 1999, 2004). Despite the disparity in methodologies and outcome variables, this suggests a trend that HL, as a neuropsychological phenomenon, plays a key role in the functioning of the immune system in both health and sickness. However, the critical methodological limitations of this set of studies necessitates caution; and it is immediately apparent that research in to this relationship should be conducted with more control for confounding variables before any resolute conclusions can be ascertained. Importantly, the findings reviewed here suggest one direction; namely that the left hemisphere is immunopotentiating, the right is immunosuppressing. Although the mechanisms of this directionality cannot be ascertained by current knowledge; one possibility is by means of interhemispheric inhibition (IHI). IHI is thought to mainly take place via the corpus callosum (Geffen *et al.*, 1994; Sullivan, 2004), and could explain the changes in immunoregulation following cerebral trauma such as stroke or surgery.

The mean effect size (r=0.536) for the HL-immune relationship determined on the basis of the studies included here was large. Two of the nine studies studies reported mean effects in the upper quartile (r>0.65) (Ivashkova *et al.*, 2002; Tarkowski *et al.*, 1998). These studies were both from the quasi-experimental/experimental category, which provides some encouragement for this relationship in the context of the many methodological flaws of this study set. The mean effect size (r=0.503) for those

studies that described a relationship between poorer right versus left functioning and increased immunity (Davidson *et al.*, 1999; Tarkowski *et al.*, 1995; 1998) was higher than the mean effect size (r=0.374) for the studies observing the opposite relationship (Dziedzic *et al.*, 2003; Kang *et al.*, 1991; Koch *et al.*, 2006). We then classified studies into activity versus functional measures of HL. Functional measures (2 studies alone) yielded an effect size of 0.63 while activity measures (8 studies) yielded an effect size of 0.515. The studies in the present review do show a high proportion of "large" effect sizes. However, attempting to compare studies that have such different independent and dependent variables, methods of data collection, methodological design and samples, can often cloud the main findings due to their disparities – and so these must be viewed with caution. Thus, we chose to focus on overall effect sizes, which can provide a standardised means of elucidating combined findings, by providing a combined perspective of the data. The fact that 55% of the effect sizes showed "large" effect sizes in the same direction is perhaps the most promising finding of the combined results, however it should be noted that many of the studies had small sample sizes – a factor which is known to increase effect size (Givens *et al.*, 1997). This indicates that despite the differences in methodology, the relationship between left-HL and enhanced immunity can be observed even under less than ideal conditions.

Concerning quality assessment, there were three studies that received observed scores in the upper quartile (6-10) (Davidson *et al.*, 1999; Dziedzic *et al.*, 2003; Kang *et al.*, 1991). These studies are of both cross-sectional and experimental design. The common factor amongst these studies is control for third variables from at least two of the designated criteria. All three of these more methodologically rigorous studies supported the conclusion that left-HL is related to immune potentiation. The mean quality assessment score (7.9) of all studies was under 50% of the possible score, which suggests that methodology in this subject area is in need of improvement. The main areas that need improvement are control over third variables and inferential validity. More control is required to ensure that the HL-immune relation does not result from variables involving health behaviour (e.g., smoking), gender or co-morbidities known to affect the immune or CNS systems (e.g., arthritis, infections, early dementia). Attention should also be paid to the immunological outcome

measures, and the reasons for choosing them. With regard to the conclusions, the main areas of improvement are the contextual evaluation - where each study fits amongst the current literature, and future theoretical and clinical implications.

Possible mechanisms underlying the HL-immune relationships

Cortisol does not appear to mediate or moderate the association between HL and immunity (Kang *et al.,* 1991; Meador *et al.,* 2004). This could mean that the HPA-axis, at least as indexed by cortisol, does not play a role in the HL-immune relationship. There are also established relationships between HL and the stress response, with the right prefrontal cortex being associated with the modulation of the stress response (Cerqueira *et al.*, 2008; Lewis *et al.*, 2007; Sullivan, 2004). An alternative mechanism to explain the HL-immunity relationship may involve the sympathetic nervous system (SNS) since provision of beta-blockers reduced the HL-immune relationship in rats (Moshel et al., 2005). There are also suggestions that there is hemispheric specialisation in autonomic control of the heart in humans, with the right hemisphere exerting sympathetic control and the left parasympathetic (Wittling *et al.*, 1998). Future studies are needed to replicate and extend these findings, and must test the functional and health implications of such mediation.

Clinical implications

The study by Gruzelier *et al*. (1996), though with several limitations, shows that HL may be related to immunity in HIV. A more recent study found that right-HL predicted symptoms of upper respiratory tract infections, independent of multiple confounders (e.g., age, sex, IQ; Gidron *et al.*, 2010). Both studies demonstrate that the HL-immune relationship has implications for immune-related diseases. This requires further research concerning both prediction of disease risk, prognosis and possible prevention using brain stimulation of the left-PFC for diseases originating from immune-suppression. The extent to which such illnesses may be prevented or ameliorated by left-PFC stimulation has important implications to understanding neuroimmunomodulation of diseases and to opening new therapeutic approaches that need to be tested. The study by Clow et al. (2003) using rTMS may be one promising

method for further investigation in relation to disease prevention. Yet, more sound research is needed to solidify the scientific ground for such interventions.

Some research has uncovered an asymmetry in both peripheral immunity and in diseases. An asymmetry in peripheral cell-mediated immune diseases has been observed in a left-sided greater prevalence of herpes zoster presentation (Dane, 2009), as well as a greater left-sided reaction to bilateral tuberculin skin tests (Dane *et al.* 2001). This peripheral cell-mediated asymmetry has also served as an explanation to findings of overall greater right-sided metastases in some gynaecological cancers (Borecki *et al.*, 2007), as well as a higher prevalence of right-sided metastases in malignancies originating on both sides of the body (Borecki *et al.*, 2007). It has been suggested that excessive left side immune reactions may be responsible for controlling left sided metastases, therefore increasing the prevalence of right side spread (Borecki *et al.*, 2007; Dane *et al.*, 2008). However, inconsistencies have been found as well when investigating paired organs. In a study that included over a quarter of a million cancer patients, Roychoudhuri *et al.* (2006) found lung and testicular cancer to have a right-sided prevalence, whereas breast cancer was suggested to be more common on the left (Roychoudhuri *et al.*, 2006). There was very little difference observed bilaterally in kidney and ovarian cancer incidence, however five year survival was shown to be higher in women with left-sided ovarian cancer than those with right-sided tumours (Roychoudhuri *et al.*, 2006). The discrepancy of the overall trend represented by breast cancer was theorized to be due to behavioural and diagnostic reasons, insomuch as the right-handed majority may be more aware of changes in the ipsilateral breast, or that right handedness may cause more movement in the breast, or preference in breast feeding, and therefore affect cancer risk (Roychoudhuri *et al.*, 2006). The extent to which peripheral immune laterality, whether related to cerebral HL or not, is responsible for such laterality in disease risk, needs further investigation.

Summary & Conclusions

This review outlines 11 studies concerning the relationship between HL and immune function. To the best of our knowledge, it is the first of its kind. It is predominantly apparent that more research is required in this area to further elucidate findings, and uncover more aspects of this relationship. Further investigation into the specific areas

of the brain, as well as lateralisation effects (nature, duration, development, etc.), and their underlying mechanisms, is also clearly needed. The role of the SNS as well as neurotransmitters (e.g., acetylcholine, dopamine) in the HL-immunity relationship needs to be examined. The present literature also brings about interesting questions for neuroimmunology. For example, given the suggested differential immunomodulation by left versus right HL, could lateralisation predict the onset or prognosis of immune-related illness? The evidence from Gruzelier *et al. (*1996) and Gidron *et al.* (2010) indicate this may be possible. If such a relationship were discernable, then it would be reasonable to suggest that health may be improved by intervening in an unfavourable lateral balance, such as via rTMS (Clow et al., 2003). Furthermore, it is possible that neuropsychological interventions could be devised and tested, to see whether they prevent or ameliorate the effects of chronic immune-related illnesses, as well as maintain good health in those unaffected by disease, particularly in people with poor left-HL. In a diagnostic setting, these findings could prove pertinent. Many chronic and life-limiting illnesses, such as HIV, show widespread individual differences in subjective symptomatic experience and prognosis (Balbin *et al.*, 1999; Grant & De Cock, 2001; Mindel & Tenant-Flowers, 2001), which could be explained, at least in part, by laterality effects. Moreover, research into this area of neuroimmunology could potentially allow us to understand more about the division of labour between the two hemispheres of the brain, particularly after trauma. From studies that have examined hemispheric trauma (i.e. epilepsy surgery or stroke) we can see the relationship between increased right hemisphere activity and functioning and poorer immunity. However, it is not clear whether this is caused by the effects of right-sided superiority, or left-sided inferiority, following a left-sided lesion. IHI can explain how HL influences immunity in this dichotic manner, but more research is required in order to understand its dynamics in this setting. In order to understand the clinical implications of laterality effects, investigation into which hemisphere exerts the most influence on immunity could be of vital importance, particularly given the suggestion by Lewis *et al.* (2007) that laterality can be essentially switched in certain psychological states. Finally, could HL also partly explain variability in the effectiveness of vaccines? One study has found that higher levels of left prefrontal activation were related to a more effective antibody response to influenza vaccination (Davidson *et al.*, 2003). Such questions

remain to be addressed in the next decade of research on HL, immunity and immune-related diseases.

Chapter 4

The Role of Hemispheric Lateralisation in HIV

LATERALISATION-IMMUNITY THEORY: PRACTICAL APPLICATIONS AND THE NEED FOR DEVELOPMENT

Of the recently reviewed literature concerning the effects of hemispheric lateralisation (HL) on immunity, only one study examined the prognostic applications of the hypothesis, by examining the effects of HL on prognosis in HIV. One of the studies examined the implications of the HL-immunity theory on HIV prognosis by examining the longitudinal correlates of HL and HIV-relevant biomarkers (Gruzelier *et al.*, 1996). The Gruzelier workgroup provided the first perspective on the possible consequences of HL in immune-mediated illness. By examining the long-term consequences of HL on HIV, this study offered a meaningful application for the theory, providing a relevance to prognosis. Moreover, HIV disease course is highly variable, and the duration of each of the relevant stages of illness, as well as the overall time to mortality, varies greatly amongst individuals (Lama & Planelles, 2007; Langford *et al.*, 2007). Some of the predictors of this variability have already been identified, and extensively researched.

Amongst the factors already known to impact upon HIV prognosis, some of the most important are concerning biological and psychosocial factors, discussed previously. Of the biological factors, viral strain, dual- or superinfection, coinfection with another pathogen (viral, parasitic, and bacterial) and host genetics are some of the most implicated in accelerating HIV pathogenesis (Lawn, 2004; Spira *et al.*, 2003; Van der Kuyl & Cornelissen, 2007). Psychosocial variables that are known to impact upon HIV pathogenesis are psychological morbidity, personality and coping styles, social support and behavioural factors such as medication adherence and addictive substance misuse or dependence (Burgoyne, 2005; Cabral, 2006; Cole, 2008; Pence, 2009; Temoshok *et al.*, 2008b).

Despite the vast wealth of knowledge accumulated over the last three decades concerning these predictors of prognosis, there still exists a variance in disease course so far unaccounted for. Given the strength of the studies evaluating the relationship between HL and immunity (Sumner *et al.*, 2011), and the existence of research concerning this modulating variable and HIV progression (Gruzelier *et al.*, 1996); it is possible that some of this variance may be explained by HL.

The research by Gruzelier *et al*. (1996) found that left HL (as measured by electroencephalograph (EEG) and neuropsychological tests) was associated with better immune outcomes ($CD4^+$, $CD8^+$ T-cells) at a 30 month follow up. Furthermore, the study also described a relatively poorer prognosis (by these same measurements) in those patients with right lateralisation. However, their sample was very small (*N*=26) and was restricted to men of bisexual or homosexual orientation. Today, the largest proportion of HIV transmission across the world occurs in heterosexual activity (Grant & De Cock, 2001; Hansasuta & Rowland-Jones, 2001). The pathogenesis of HIV may depend on the biology and socioculturally influenced health behaviours of individuals, which cannot be accounted for in such a restricted sample, nor generalised to a wider population (Derdeyn & Silvestri, 2005; Gifford *et al.*, 2002; Lama & Planelles, 2007). Most importantly, Gruzelier *et al*. (1996) did not control for effects of baseline immune parameters and other confounders (e.g., education, mode of infection, other illnesses) that may affect the autonomic nervous system (ANS) (Cole *et al.*, 2003) or the course of HIV, either statistically or methodologically. Additionally, the study did not selectively recruit right handed participants, a measure which is central in HL research. Left handed individuals characterise atypical interhemispheric communication and hemispheric specialisation (Iwabuchi & Kirk, 2009; Toga & Thompson, 2003), which contributes too much heterogeneity in this measure for an initial investigation. Moreover, the research was conducted before the advent of highly-active antiretroviral therapy (HAART), one of the most significant developments in the fight against the HIV epidemic (Simon *et al.*, 2006). The study participant pool was drawn from the Zidovudine trials in 1996, and so some participants were receiving this treatment. However, this is one chemical agent in a repertoire of what now consists over 20 – a significant advance since these early trials. Whilst all of the participants were in the asymptomatic phase of HIV illness, it is still possible that some of them may have been eligible for HAART treatment were the study conducted today. All these limitations question the validity of their inferences, and the application of the findings to modern HIV patients.

In summary, although much is known about how HIV pathogenesis is modulated, there still exists disease course variance that is unexplained. A promising line of research for exploring additional prognosis factors is HL, and has already been tested in a small sample of patients. The results of this study showed very clear associations

between HL and prospective $CD4^+$ and $CD8^+$ T-cell count changes in HIV patients, with converse relationships being described. However, this original study was subject to a number of methodological flaws – either in statistical control or sample selection. These flaws mean that the findings are very difficult to validly generalise to HIV^+ people as a patient group. Therefore, a study was planned to advance these findings, in a larger, more representative sample of HIV patients, with stricter control for those variables identified as impacting upon HIV pathogenesis.

THE ROLE OF HEMISPHERIC LATERALISATION IN HIV-1

Objectives

The present study was developed to examine the influence of the independent variable of HL on the dependent variable of $CD4^+$ T-cell count, as a surrogate biomarker for HIV progression. The key differences to the original study by the Gruzelier workgroup are in methodological and statistical control. Methodologically, a larger sample of participants was sought from broader demographic profiles. Only right-handed participants were recruited to eliminate the heterogeneity observed in HL amongst left handed and ambidextrous people. Additionally, strict exclusion factors were implemented to reduce confounds. These factors include the exclusion of pregnant women, those patients with other illnesses known to affect HIV progression or general immune system functioning (e.g., hepatitis, cancers, psychological morbidity, autoimmune and neurological disorders), and any patients who were also currently physiologically or psychologically dependent on addictive substances. Statistically, control for baseline immunity, mood, health behaviours and socio-demographic variables were exacted to account for the impact these factors may have on the outcome. Potential confounding variables in the relationship were identified *a priori* from the existing literature on HIV progression, and will be discussed in detail later.

The aims of the study were to 1) replicate and advance the findings of the Gruzelier workgroup using stricter methodology and a more representative sample of HIV^+ patients; 2) identify and evaluate potential variables that may moderate or mediate the relationship between HL and HIV prognosis; and 3) explore the data to analyse the contribution of HL to HIV-relevant behaviours.

Hypotheses

The major hypothesis of the present study was that HL would predict prognosis in HIV, as indicated by differential decline in $CD4^+$ T-cells prospectively. This relationship was to be examined by controlling for baseline immunity, non-adherence to HIV medication and mean time between immunological assessments. Further, as a secondary (or minor) hypothesis, it was anticipated that left HL would predict a better prognosis, and right HL a worse prognosis.

Chapter 5

Materials & Methods

PARTICIPANTS

Sample

The participant sample was drawn from a clinical population of HIV^+ outpatients of the Universiteit Ziekenhuis Brussel (UZB), Belgium which is a hospital associated with the Vrije Universiteit Brussel. The hospital is a predominantly Flemish speaking organisation; however it services patients of all demographics within the local community of Jette and the surroundings near Brussels. HIV^+ patients attend the internal medicine clinic as outpatients, at all stages of illness from initial diagnosis to long-term surveillance and monitoring. Consequently, a broad profile of patients was potentially available for candidacy.

A total of 74 candidates were put forward for study inclusion by clinicians, using the inclusion criteria outlined below if the patients indicated willingness to participate. Of these, 72 became full participants, with two candidates being excluded on medical grounds (one had a diagnosis of cerebral toxoplasmosis, the other was diagnosed with a Hepatitis virus and was receiving Interferon treatment). Recruitment spanned a three month period. After full follow-up data had been collected, 68 participants remained with sufficient data to carry out analyses.

Sample Size Calculation

Preliminary power calculations provided a target sample size of between 64 and 74 participants. This sample size was calculated following two assumptions; 1) a total of two neuropsychological and six background variables will be predictive of immune outcomes ($CD4^+$ T-cell and viral load counts); and 2) assuming a statistical power of 0.80, a statistical significance of $p<.05$, and a contribution of 9% of the variance by neuropsychological tests to immune-outcomes, beyond the contribution of 30%-40% by the background measures (Cohen & Cohen, 1983).

General Recruitment Criteria

General guidelines for candidacy were to recruit right-handed adult men and women between the ages of 18 and 70 years old, in good general health. All candidates were to have a formal diagnosis of HIV, but not AIDS. Doctors were advised only patients with a $CD4^+$ T-cell count of over 200/mm^3 should be approached for candidacy. This rationale was implemented to ensure cognitive capability, as HIV is more likely to have infected the brain at this stage of disease, and because $CD4^+$ T-cell loss

accelerates beyond this threshold (Weber, 2001). Only right-handed participants were required (discussed below), with a good level of literacy in French, Dutch or English. In order to provide true informed consent, candidates were required to have a good pre-assessed level of comprehension and cognition. Doctors were advised not to approach patients who had known cognitive deficits, or were otherwise similarly vulnerable. Cognitive capability was screened for in all participants, see below for further details. Patients were not offered an incentive (financial or otherwise) for participation, and were advised that the interview would take between 15 and 25 minutes. Doctors at the Internal Medicine HIV clinic approached suitable candidate patients, and referred them to participate. Suitability was determined by the inclusion and exclusion criteria outlined below.

Inclusion Criteria

Eligible candidates for the study were those patients of UZB who were HIV^+ and had not been diagnosed with an AIDS-defining condition at the time of study entry. Once the illness has developed to AIDS, neurological complications are prevalent which could corrupt the neuropsychological findings and impair cognition (Grant & De Cock, 2001; Hansasuta & Rowland-Jones, 2001; Simon *et al.*, 2006). End-stage AIDS is characterised by multiple opportunistic infections, and renal and hepatic failure; all of which could alter the immune profile of the patient, thereby potentially obscuring the $CD4^+$ T-cell and VL trajectory (Fine *et al.*, 2008; Welch & Morse, 2002). Initially only patients not taking a HAART regimen were sought, in order to rule out medication effects. However, due to relatively slow study uptake and time constraints, it was decided to include HIV patients taking a HAART regimen.

Only right-handed people were selected for recruitment. It is known that left handed individuals exhibit atypical inter-hemispheric communication (Iwabuchi & Kirk, 2009), and atypical hemispheric specialisation (Toga & Thompson, 2003), which may interfere with the indices of HL. It has also been suggested that functional and biochemical cerebral asymmetries may be less pronounced in left-handed individuals (Previc, 1996; Zhavoronkova, 2000).

In order that the participants were able to fulfill all of the study criteria, they were also required to read, speak and write a good level of Dutch, French or English. Participants were advised of their language options for carrying out the study, and

were advised to conduct the study using the language with which they felt most comfortable.

Exclusion Criteria

The exclusion criteria for the present study were based on three research requirements. Firstly, as full informed consent was required, cognitive capability screening was conducted to ensure the candidates were functioning to a minimum (but high) standard, with a cut-off score applied to the screening (see below). Secondly, as the study examined factors relating to prognosis in HIV, a number of extraneous factors, which are known to affect the pathogenesis of HIV beyond its natural course, were identified *a priori* for exclusion. These factors are listed below.

Finally, as the research focussed on the functioning of the brain and its relationship to disease prognosis; a number of factors that are known to interact with the relationship between the brain and the immune system required identification. It should be noted that some factors that relate to HIV disease course also relate to the relationship between the brain and the immune system. The main exclusion measures are now described and explained below.

Cognitive Capability

Candidates were assessed for cognitive impairment using the Mini Mental State Examination (MMSE) (Folstein *et al.*, 1975). This test is a widely-used cognitive screening tool with good specificity for identifying dementia and mild cognitive impairment in a variety of patient samples (Mitchell, 2009). The MMSE has a total score of 30. It is suggested that those exhibiting no cognitive impairments will score between 25 and 30, those with mild to moderate impairment 18 to 24, and those with severe impairment will score below 17 (Folstein *et al.*, 1975). There has been some criticism levelled at the MMSE in being able to confirm a diagnosis of impairment, however a meta-analytic review has suggested that it is a valid tool for ruling out these cognitive deficits in a variety of samples (Mitchell, 2009).

In order to avoid inclusion of those patients who may be even slightly cognitively impaired, the cut-off score of 25 was adopted. Any participants that attained a score lower than 25 were advised they would not be able to continue with the study. The MMSE was available as a standardised version in all three study languages and so no additional translation was required.

Factors Relating to Atypical or Accelerated HIV Pathogenesis

Clinical morbidity of psychiatric illness is known to increase the pathogenesis of HIV, exacerbate HIV related symptoms, and impact on the adherence to HIV treatments (Evans *et al.*, 1997; Ickovics *et al.*, 2001; Mijch *et al.*, 1999). Any candidates who had received a clinical diagnosis of a psychological or psychiatric disorder (such as major depressive disorder, bipolar affective disorder, schizophrenia, personality disorder, anxiety or dementia) were excluded from participation.

Current dependence on addictive substances, both illicit and legal or prescription can cause earlier onset of AIDS-defining conditions in the HIV^+ patient (Basso & Bornstein, 2000), and therefore those candidates who were classed as dependent on any addictive substance were unable to participate.

Factors Relating to Atypical Brain to Immune Communication

Historic dependence to and abuse of alcohol, cocaine and methamphetamine in HIV^+ patients has been associated with neurological interference and decreased cerebral functioning (Basso & Bornstein, 2000; Chang *et al.*, 2005; Jernigan *et al.*, 2005; Pfefferbaum *et al.*, 2007; Schulte *et al.*, 2008). Any such neurological interference would potentially have affected the measures of lateralisation (HL), and so any participants reporting such dependencies were excluded from research.

Autoimmune disorders such as arthritis and systemic lupus erythmatosus were screened for as they are indicative of atypical immune system functioning. Equally, patients with a chronic medical condition that significantly influences the normal functioning of the immune system, such as cancer, cardiovascular diseases, respiratory disease, glaucoma, or any form of hepatitis were also excluded.

Medications administered for less severe, and perhaps acute rather than chronic, medical conditions have modulating effects on the autonomic nervous system (ANS), which in turn is suggested to modulate the functioning of the immune system (Bellinger *et al.*, 2008; Quan & Banks, 2007; Wrona 2006). Patients prescribed sympathomimetics, anxiolytics, antihistamines or steroids were therefore excluded from candidacy consideration.

In women, pregnancy is known to create atypical functioning of the ANS (Pickel *et al.*, 2008) and so pregnant women were not invited to participate.

Any pre-existing medical condition or incident which would have resulted in clinically meaningful damage to the brain would not only interfere with the

relationship between the brain and the immune system, but could also have affected the neuropsychological assessment of HL. Patients with history of traumatic brain injury, surgery to the head or brain or who had suffered any periods of cerebral hypoxia were excluded.

MATERIALS

The independent variable of HL was assessed with two neuropsychological tests, and a number of other instruments were implemented to assess other factors identified as being associated with HIV disease progression. In order to isolate the effect of HL on the dependent variables (immunological and virological outcome), variables identified previously in the literature as being associated with these outcome variables were measured to provide confounder and moderator assessment. These items are listed below, along with the methodological considerations surrounding their selection and purpose. Copies of all instruments can be found in Appendix B.

Neuropsychological Measures

To assess HL, two neuropsychological tests were used. Two tests were selected due to the associated problems involved in collecting neuropsychological data on lateralisation (discussed below). It was decided that two assessments that index laterality in different manners could provide a means of inter-test reliability should the two indices produce different effects, by assessing correlation between the data from the two. Both tests have strengths and weaknesses, and it was hoped that by using two the conceptual strength of each assessment would be bolstered.

Neuropsychological tests like the line bisection task (LBT) require visuospatial negotiation, as well as motor skills in physically completing the task. The task itself is supposed to utilise the right side of the brain, at least in right handed individuals (Flöel *et al.*, 2005; Foxe *et al.*, 2003), and as such may present a left-sided bias (*pseudoneglect*), which is observed more strongly in women (Çiçek *et al.*, 2003; Milner *et al.*, 1992). As the purpose of this thesis was to examine overall HL of activity, rather than HL of specialisation, examining just this aspect of HL was considered to be a potentially insufficient composite of HL.

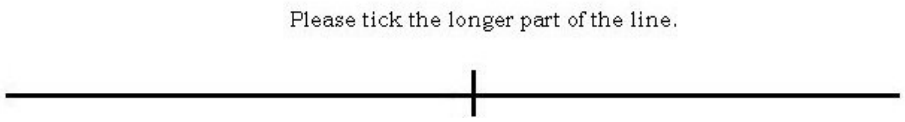
Questionnaire-based assessment of HL such as that levelled by the Hemispheric Preference Test (HPT) (Zenhausern, 1978) can provide a way to overcome operational cognitive bias. The HPT assesses laterality by means of surveying the

respondent's behaviours and cognitions as opposed to relying on perceptual and attentional processes. However, there are also affective states which correlate with the responses of the HPT, with suggestions that the scale relates directly to anxiety (Russo *et al.*, 2001), phobia and depression (Merckelbach *et al.*, 1990).

Line Bisection Task

The second measurement of HL was conducted by a computerised online version of the Line Bisection task (LBT) (Milner *et al.*, 1992). The test was held on a secure server with password protected entry (within www.brunelhivresearch.org). This task presents participants with a series of pre-bisected lines for a period of one second; the participant must then decide whether the line bisects more to the left or right. This task also correlates with resting EEG activation asymmetry (Nash *et al.*, 2010). The current format of this test provided ten genuine and five randomly-generated sham trials, with presentation order randomised for each participant. Sham trials were very obviously bisected to one side; genuine trials were bisected exactly at the median. An example of a genuine trial and response entry are provided in figures five (a and b).

(a)



(b)

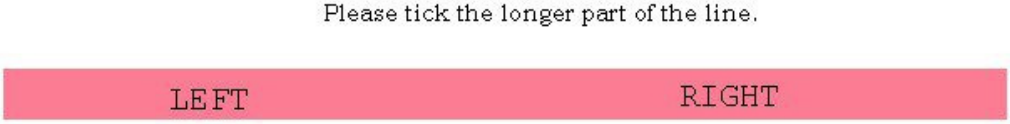


Figure 5. Examples of the computerised LBT trials.
Above shown are: (a) a genuine LBT trial; and (b) a trial response input.

Hemispheric Preference Test

The HPT (Zenhausern, 1978) is a 20-item self-report test that measures the amount of preference for either right hemisphere-based cognitions (e.g. "How vivid are your dreams?") or left hemisphere-based operations (e.g. "How quickly do you read?"). The respondent answers to their individual preference and an index of lateralisation can be ascertained from those answers.

This inventory is scored on the basis of one to ten for each item of the scale. However, due to an error in encoding the first five participants were scaled only on one or ten. A further ten participants were administered this questionnaire with a one-to-six scale for response. The remainder of the participants were surveyed with the correct one-to-ten scale. Adjustments for these errors were made in statistical analysis, detailed below.

This scale has been validated against EEG measures of hemispheric activation (Genovese, 2005; Merckelbach *et al.*, 1997). This test was developed originally in the Dutch language, and an English version already existed; thus only one translation (French) was required.

Immune Parameters

The HIV-specific immune parameters being assessed were $CD4^+$ T-cells and viral load (VL) counts, in both their whole and $\log_{10}$ form. VL was available in both formats, and so was collected with both formats. Clinicians often collect the VL data in both formats in order to describe the change of VL between measurements. Because log conversions allow the recoding of very large numbers in to more small, manageable figures, it is often easier to assess the significance of VL change using the log expression - with a change of at least 0.5 $\log_{10}$ being classed as a clinically significant change in HIV medicine (Ginocchio, 2001). Although both figures are essentially expressing the same value, it was decided to use both as they are incrementally different, and one may provide more sensitivity to change within this context.

International guidelines for the monitoring of HIV disease are that patients should undergo immunological and virological survey testing every three to four months (AIDS Education & Training Centers National Resource Center, 2009); however this is dependent upon the patient's own vigilance of their health. Participants were advised that their immunological and virological data were to be collected from their medical records, and therefore did not require any additional serological testing. Data collection of immune parameters was conducted three months after the last participant had completed their baseline interview. Three points of immunological and virological data were sought, spanning the longest period of time where possible.

Mood

In order to assess levels of anxious and depressive mood the participants were given the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983). The HADS has been validated in a self-report format in factor structure, item analysis and internal consistency (Mykeltun & Stordal, 2001). Many other scales used for the assessment of anxiety and depression rely on the measurement of somatic variables, whereas the HADS does not. HIV and its treatment create many of the somatic symptoms also present with depression and anxiety (e.g. restlessness, nausea, anorexia) (Farinpour *et al.*, 2003; Ickovics *et al.*, 2001), so assessment of anxiety and depression in the HIV^+ patient must be done without relying on somatic symptomatology. In the HIV setting, the HADS has been found to have "excellent" internal consistency, convergent validity and test-retest reliability (Savard *et al.*, 1998). The scale has also been assessed using symptomatic and asymptomatic HIV patients, and has been suggested to be unaffected by the presence of HIV symptoms due to its reliance on cognitive-affective (as opposed to somatic) symptoms, particularly in reference to depression (Savard *et al.*, 1998).

This scale was obtained in standardised forms in French, Dutch and English and so no translation was required.

Medication Adherence

Treatment of HIV involves the implementation of a regimen of highly active antiretroviral therapy (HAART), which can be administered and altered in accordance with many different factors (Grant & De Cock, 2001; Harris & Bolus, 2008). The present study included patients both HAART-naïve (N=20) and HAART treated (N=49). In HIV^+ patients, treatment adherence is a key issue to pathogenesis.

The Morisky Medication Adherence Scale (MMAS) (Morisky *et al.*, 1986) is a four-item scale, which asks respondents to indicate a yes or no answer to the questions and also considers reasons why participants may not adhere (e.g., since they feel better). The scale is negatively weighted, so in order to confirm a positive behaviour, a negative response is given (e.g. Do you ever forget to take your medicine?). It was designed purposefully to overcome what the developers term a "yes-saying bias", as patients are most used to answering questions that require an affirmative answer to indicate compliance (Morisky *et al.*¸ 1986). This scale has been validated in HIV^+

population samples (Pratt *et al.*, 2001). This scale was only obtainable in English, and so required translation in to both French and Dutch.

Health Behaviour

General Health Behaviour

Some health behaviours have been found to be particularly relevant to HIV pathogenesis and general health in the HIV^+ patient. Due to the associated interactions of drug use and HIV pathogenesis and HAART adherence (Baum *et al.*, 2009; Cabral, 2006; Feldman *et al.*, 2006; Pfefferbaum *et al.*, 2007), tobacco, marijuana, alcohol and cocaine use were surveyed for the last month. Candidates were initially screened for substance dependence or addiction, however casual use has less of an impact on the health of the individual, and so this information was collected to be accounted for, rather than ruled out. Participants were asked how frequently they used each substance in the last month, in the stratification of: no use, once or twice, once a week, several times a week, and daily. This survey was designed to allow exploration of potential confounding, moderating or mediating factors. The evidence is contentious as to whether these substances have a significant impact upon HIV progression, but there is sufficient evidence to suggest they may be confounding variables.

Sexual Health Behaviour

The participants were surveyed for sexual health behaviours (number of sexual partners, frequency of condom use) in the prior 12 months. Coinfection with multiple HIV strains or other sexually transmitted viruses can profoundly alter the disease course of HIV, and so condom use was assessed (Chan, 2004; Gore-Felton & Koopman, 2008). Although condom use does not necessarily indicate the presence of coinfection, it is an extrapolation of potential *risk* of coinfection – which may not have been medically apparent at the time of interview (and therefore evident to the screening doctors). This information works synergistically with the number of sexual partners the individual has and their relationship status; insomuch as the combined picture of these factors provides a more comprehensive index of risk. For example, the relative risk of coinfection of someone who reports no condom use, but is in a

relationship with only one sexual partner would be very different to someone who reports little condom use, no relationship and many sexual partners.

The participants were asked how many sexual partners they had in the prior 12 months, and how often they used condoms (always, often, sometimes, never). This scale was devised originally as a simple surveillance strategy of this type of health behaviour, but after conducting the interviews with the participants it became apparent that there were more phenomenologically relevant strata for this information. For example, there were frequent reports of no condom use, but the other circumstances that each individual reported mitigated the level of risk they apparently were prepared to engage in. These responses were then therefore re-coded according to their potential impact upon HIV progression; those responding "never" were categorised in to those with no relationship or sexual partners (thus representing the most minimal risk of multiple infection); those in a relationship but did not have sex; and those who did have a relationship or sexual partners and did not use condoms (representing the highest risk of multiple infection). This scale was thus transformed and scored in the following manner:

1 – no relationship, no sex, "never" on condom use;

2 – a relationship, no sex, "never" on condom use

3 – "always"

4 – "often"

5 – "sometimes"

6 – reported sexual activity (either within or without a relationship) and "never" on condom use.

This information was collected as part of the demographic questionnaire, detailed below.

Socio-demographic Factors

A two-part survey-style questionnaire was devised to collate information about the participants' socio-demographic background and historic information relating to their HIV^+ status. The socio-demographic section of the questionnaire documented age, gender, ethnicity, educational level, employment status, sexual orientation and relationship status– these have all been associated as being related to HIV disease prognosis (Eich-Höchli *et al.*, 1997; de la Hera *et al.*, 2004). The second half of the

questionnaire related directly to HIV, and information concerning mode of contraction, duration of illness or diagnosis, and duration and type of antiretroviral treatment. Again, all of these factors have been related to HIV prognosis (Eich-Höchli *et al.*, 1997; Kitahata, 2010; Wood *et al.*, 2003) and had been identified as some of the potential key factors of covariance for the present study.

PROCEDURE

All candidate and participant interviews were conducted in a private room in the internal medicine clinic. This room was equipped with a desk, several chairs and a desktop computer to conduct the LBT. Participants were provided with writing materials and paper questionnaires. Some patients attended the clinic with partners, and were permitted their partners presence at interview; but were advised of the personal nature of the questions and were advised to conduct the interview alone. Only one participant chose to remain with their partner for the process of the interview.

Following being put forward for candidacy by their doctor, the candidate would then be provided a detailed information sheet and consent form. If the candidate opted to participate, they were then screened using the MMSE. If the candidate scored over 25 on this item they were then considered a participant. Those that scored less than 25 were advised that they could not continue, and were thanked for their time. Participants were then verbally taken through the demographic survey questionnaire. If there were items that the participant could not answer, these were logged as a null response. Both the HADS and HPT were provided to the participant for self-completion. The participants were advised that they could ask for clarification of the questions if necessary, but that they should answer as accurately as possible. The HADS versions provided instructions for completion, and the participants were advised on how to respond to the HPT questions verbally. The required response for the HPT was to circle, or otherwise mark, one number of the likert scale that they felt corresponded to their own perspective on the question. Any participants that could not respond to just one of the multiple-choice responses for the HADS, or one of the inventories on the likert scale for the HPT were advised to indicate a group response (i.e. circling two responses); the median of this response was then recorded for analysis.

Upon completion of the study-proper, participants were provided with a contact card detailing their participant number and contact details of the main researcher. They were then invited to ask any questions they had and were thanked for their time. A flow-chart of the study procedure can be found in figure six.

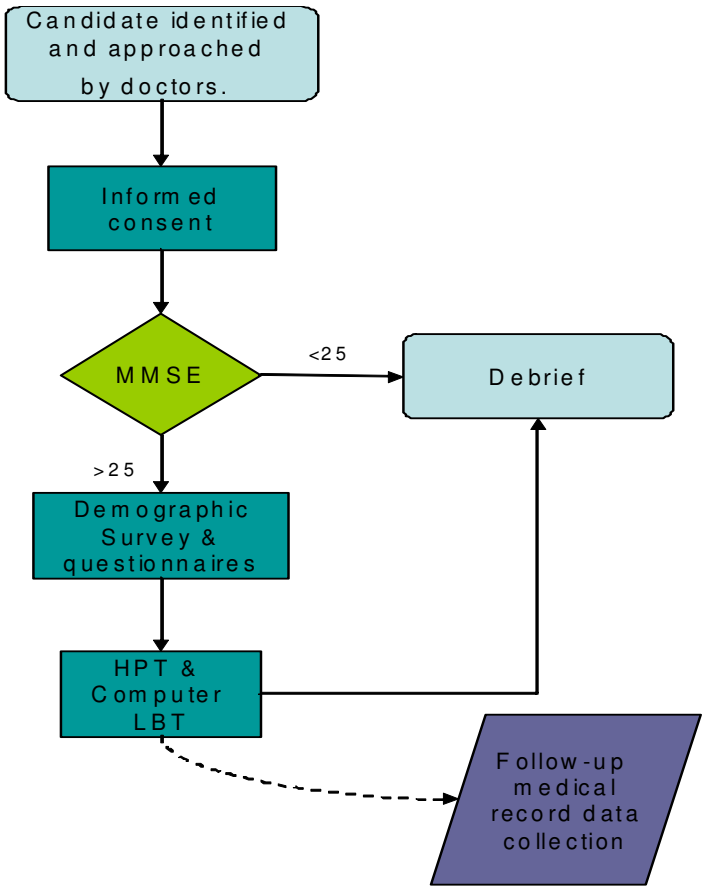


Figure 6. A flow-chart of study procedure from candidacy to completion.

Data collection was initially carried out by the principal researcher (RS). Due to time and financial constraints, the remaining data collection was conducted by two research assistants. Follow-up data collection was completed solely by the principal researcher. The research assistants were suitably qualified for and experienced in the collection of psychological data and suitably adept with all three languages being used. The principal researcher coached the research assistants in the procedure, and assistants were advised of potential procedural problems and how to overcome them (e.g. participants becoming distressed or wishing to terminate participation). Two participants became significantly distressed during data collection. At the point of the distress becoming apparent the interviews were suspended and the participants were counselled in relation to their rights to withdraw. Both participants were repeatedly

offered the opportunity to discontinue participation; however they both decided to continue with the interview after a brief period of suspension.

Follow-up Data Collection

Follow-up data was collected using the computerised medical records held at UZB. Information collected at this juncture was $CD4^+$ T-cell count and VL; and the dates at which the patient attended the clinic for these readings. Whilst the attendance of these clinics is guided by the clinicians, it is ultimately the patient's responsibility to return and so the frequency of return visits varied. As not all patients attended the clinic with the same frequency, time points for which there were data for both immune (T-cells) and virion (VL) cell were prioritised over duration of time between visits. This was prioritised in order to preserve these cross-sections of immunological status to provide discrete "snapshots" of the disease course. In some instances there were only data for either immune or virion cell data; which could skew data and potentially make the analysis of visit frequency (a health behaviour in itself) redundant. Immunological data was collected for three time points; an artificial baseline, one clinic visit retrospective to the original baseline data collection (T1); first follow-up which corresponded with baseline interview (T2), and second follow-up one clinic visit prospective to the original baseline interview (T3). Time-points were sought approximately three months apart where possible (i.e., true baseline (T2) ≈ at interview; artificial baseline (T1) ≈ T2-3months; follow-up (T3) ≈ T2+3 months). In the case of new patients, who had not had immunological surveillance before the interview, only prospective data was collected. As the frequency of clinic visits varied between participants a mean was taken of the time between clinic visits, so that it could be controlled for in statistical analysis.

DATA CLARIFICATION

One participant represented an outlier for the HPT variable. Using scatterplot analysis of the correlation distribution between HPT and $CD4^+$ T3 it became apparent that this participant was heavily skewing the distribution of the HPT data and the pattern of this correlation. Therefore it was decided to exclude this participant's data for the entirety of the data analysis.

Three participants were demographic outliers. The majority of participants were of European or African descent, aside from one who was of South American extraction.

Equally, the majority of participants reported themselves to be either homosexual or heterosexual, but two reported being bisexual. In order to include these outliers in covariable and moderator analyses it was decided that they be adjusted to fit in to one of the larger groups. This decision was made in order to retain the maximum number of participants for analyses, as the sample (N=68) was within the minimum limits delineated by the *a priori* statistical projection (N=64-74). This adjustment was done by the means of analysing each participant's specific demographic information and aligning them to the group that they corresponded to in other demographic characteristics (e.g. age, gender, sexuality, education level and employment status). The South American participant was included in the European group. One bisexual participant was sorted to the heterosexual group, the other to the homosexual group. Data analyses were conducted with these participants in their unsorted and re-sorted groups to ensure there was no significant change in effect size for the main analyses. As these analyses were unaffected by the resorting of these participants, they remained in their re-sorted groups.

Those participants with missing data were still included provided there were sufficient reported data for the independent and dependent variables. If participants were not definitive with their answers on continuous variables, and instead supplied a range or selected two responses to an inventory question, a mean value was recorded.

ETHICAL CONSIDERATIONS AND APPROVAL

Informed consent and debrief protocols were designed in line with British Psychological Society code of ethics and conduct (British Psychological Society, 2006). All methods, materials and instruments were disclosed to and approved by the Brunel School of Health Sciences and Social Care Research Ethics Committee, as well as the Vrije Universiteit Brussel/UZB Medical Research Ethics Committee, where the study took place. Any amendments to protocol were also subject to ethical approval by each of the stated committees. Examples of ethical clearance notifications and amendment approvals can be found in Appendix A.

Due to the nature of recruitment (referral by Doctor) extra care was taken to ensure candidates were ensured of their rights in voluntary participation, with relevance to potential coercion that could be inherent in being selected by an authority figure such as a clinician. A section was added to the informed consent information sheet explicitly stating that their participation, or declination to participate, would have no

bearing on their access to or standard of care from the Hospital and its staff. An example of the participant information sheet can be found in Appendix B. In Belgium, where patients were recruited, doctors see it as their ethical and clinical responsibility toward patients to support recruitment into scientific studies; hence they and not the research staff recruited patients.

Medical record data retrieval was collected using the participants' patient numbers (in an alphanumeric code), provided by the head of the Internal Medicine department at UZB. This request was made for all candidates put forward by the clinicians, in order to maintain confidentiality in those who declined to participate or required study exclusion. A key linking participant names and their patient number was retained by the principal investigator (RS) only, to ensure anonymity and in the event of a withdrawal request. This data was collected by the principal investigator from UZB's computer systems, which are held in the Dutch language, using only the alphanumeric patient code. Due to the investigator's limited familiarity with the Dutch language, it would not have been possible to examine any other parts of the participants' medical files. The clinician provided training with the computer system to obtain study-relevant data only ($CD4^+$, VL). Participants were advised from the outset of the study in the information sheet (Appendix B) that all members of the research team were bound by confidentiality and only the $CD4^+$ and VL data would be sought by the investigator. The collection of this data was undertaken in a private room within the clinic.

LANGUAGE CONSIDERATIONS

All scales were initially obtained in English. In order to cater for a multi-lingual population, all instruments were sought in standardised forms in French and Dutch. If scales could not be obtained in an official standardised version for the relevant language, translation was carried out. Those materials that were translated from English were either done so, or were validated by, a native speaker of that language.

The participant interviews at baseline were conducted in the participant's native language where at all possible. There were two participants whose native languages were not French, Dutch or English (one Scandinavian, one South American participant), and they were advised that they could complete the study in whichever of the three study languages they felt most comfortable with, or were advised that they should not participate if they were not sufficiently proficient in any of these

languages. Both participants chose to continue with the study; one chose English (the Scandinavian participant) and the other chose French.

STATISTICAL ANALYSIS

HL and immunity

In order to assess the main relationship between HL (HPT and LBT separately) and the outcome measure of $CD4^+$ T-cell count (T3); a linear multiple regression analysis was used. All data analyses were run using the calculated left hemispheric lateralisation index (LHL) of the Log_{10} transformation of the HPT, and a corresponding LHL index of the LBT. A "left" index of the HPT was calculated using the formula LHL= 100 x (L-R)/(L+R). The errors in encoding for the HPT data were overcome by excluding the first five participants (coded 1 or 10), and then performing z score transformations for the remaining data set in order to standardise the one-to-six and one-to-ten scores. The data from the LBT were uncorrupted, and so analyses including this variable were conducted with the full participant sample. Due to data skewness and kurtosis present in HPT data, both square-root and log_{10} transformations were tested. The log_{10} transformations presented the least skewness and so were employed for the analyses; however there was still significant skewness apparent in this variable (see Appendix C). Additional analyses were conducted using Spearman's *rho* correlation in order to counteract bias due to outliers within the dataset. To ensure that one of the key objectives of the study – to control for confounding variables – was preserved, and since the Spearman's test does not permit controlling for confounders, the T3 $CD4^+$ scores (dependent variable) were residualised on T1 $CD4^+$ data and medication variables.

Confounding variables

Potential confounding variables for the main data analysis were identified from the literature *a priori*, for both the outcome and independent variables. Those variables that were identified from the literature as being potential confounding variables for HIV variables were baseline immunity ($CD4^+$ T-cell count at artificial baseline), HAART use, HAART adherence and duration of HIV infection, as all of these variables have the capacity to significantly alter, or predict, outcome $CD4^+$ T-cell count (Deeks, 2006; Pratt *et al.*, 2001; Simon *et al.*, 2006).

A set of subsequent tests were conducted to identify true, statistically confounding variables identified in the present sample. The significance of each potential confounding variable was assessed against the outcome variable ($CD4^+$ T-cell count at T3) using bivariate Pearson's correlation (continuous data) and *t* test or ANOVA (categorical data). Those variables that presented significant ($p<.05$) associations were used as covariates in each multiple regression or correlation.

Moderators

Moderators were identified observationally (ethnicity) and through methodological contingency (HAART treatment). It became apparent during data collection that the sample comprised of two groups: male, homosexual Europeans, and female, heterosexual Africans. This clinical separation is similar to that observed in other studies (Anastos *et al.*, 2000; Jarrin *et al.*, 2008; Smith *et al.*, 2007), yet its implication in immune outcomes has not yet been analysed in such a way. Analysis of HAART as a moderator served a methodological purpose, to understand whether the inclusion of a mixed-treated sample would modify the potential results. Further, as HAART is a immunomodulating medical treatment, it is possible that its presence may attenuate, or even abrogate, the effects of HL on immunity in HIV^+ patients. Additionally, the investigation of the moderation of HAART in this context presents clinical significance to the interpretation of the findings. Analyses comparing sub-samples were undertaken using *t* tests for continuous data and X^2 tests for categorical data. As splitting the sample according ethnicity and medication resulted in smaller sample sizes for the main analysis Spearman's *rho* correlation tests were used to assess the HL-immune relationship for each separate sub-sample.

***Post-hoc* tests**

Exploratory analysis of behavioural data was conducted in the split dataset using two-tailed bivariate correlations. Both measures of HL (HPT and LBT) were employed to fully explore the available data. Exploratory analyses were run in both the whole sample and the ethnicity-split subsamples to ascertain further implications of the moderator.

Statistical tools & parameters

Statistical analyses were conducted using SPSS Statistics (IBM SPSS, version 17.0), with p values <0.05 indicating statistical significance. Raw data output can be found in Appendix C.

Chapter 6

Results

PARTICIPANT SUMMARY STATISTICS

The original sample size was 72, and four participants had to be excluded from final analyses. Three of these participants were excluded due to comprehension problems, which became apparent during interview, the other was excluded due to outlying HPT scores (see previous chapter).

Of the remaining participants several demographic outliers required re-classification in order to maximise the dataset potential. As outlined in the previous chapter, one non-African non-European participant, and two bisexual participants were re-classified into other subcategories in accordance with their other demographic characteristics. Each of these participants was compared to the other profiles of participants (either by ethnicity or sexuality) and was re-sorted to the category that fit their other reported variables. This reclassification was conducted as both ethnicity and sexuality could have potentially been important confounding or moderating variables, and these participants would have been lost in those analyses without reclassification.

Table 4. Descriptive statistics of continuous data with mean, standard deviation and number of respondents.

Characteristic	Mean	SD	*N*
Age (years)	43.30	8.95	68
Duration of HIV (years)	6.32	6.36	64
MMSE (maximum range 25-30)	29.03	1.25	68
Mean period between clinic visits (days)	109.97	33.98	65
Non-Adherence (maximum range 0-4, 4 indicating maximum non-adherence)	.55	.77	47
Number of sexual partners (prior 12 months)	7.28	13.38	68
HADS Anxiety (maximum range 0-21)	7.22	4.37	68
HADS Depression (maximum range 0-21)	4.13	3.54	68
Line Bisection Index of Left Lateralisation (maximum range 0-10)	5.46	2.64	68
HPT Left Lateralisation Index Z Score (based on log transformation)	3.05	.073	61

Sample Description

The full participant dataset comprised 68 individuals (50 male, 18 female; mean age= 43.30, SD ±8.95 (years)). The mean self-reported duration of illness was 6.32 years (SD= ±6.36 years), with just over 70% (*N*=48) currently taking a HAART regimen. The majority reported contracting HIV through sexual contact (*N*=43). Within this dataset the majority were of European extraction (*N*=45), were homosexual (*N*=38),

were not in a relationship (*N*=33), had attained an academic level of education (*N*=35), and were employed full time (*N*=41). Non-adherence was low amongst those taking a HAART regimen. The dispersion of the HL scores was very large. Before z score transformations were conducted, the dataset showed a majority of left lateralised participants (i.e. scoring higher on "left" versus "right" items).

Full details of summary statistics can be found in tables 4 and 5.

Table 5. Descriptive statistics of categorical data with number of respondents and percentage of total respondents for that category.

Characteristic		***N***	***%***
Gender	Male	50	73.5
	Female	18	26.5
Ethnicity	European	45	66.2
	African	23	33.8
Education	Primary	5	7.5
	Secondary	23	34.3
	Vocational	4	6.0
	Academic	35	52.2
	Missing/Undisclosed	1	
Employment Status	Employed full time (FT)	41	60.3
	Employed part time (PT)	4	5.9
	Homemaker/Full time parent	1	1.5
	Unemployed due to health	3	4.4
	Unemployed	17	25.0
	Retired	2	2.9
Sexual Orientation	Heterosexual	30	44.1
	Homosexual	38	55.9
Relationship Status	Single	33	49.3
	Married/Cohabiting	11	16.4
	In a Relationship	23	34.3
	Missing/Undisclosed	1	
HAART* Medication	HAART treated	48	70.6
	Untreated	20	29.4
Mode of Contraction	Sexual Contact	43	64.2
	Injection Drug Use	1	1.5
	Medical Contact	5	7.5
	Unknown	18	26.9
	Missing/Undisclosed	1	
Condom use (12 months)	Never – No relationship, No sex	10	14.9
	Never – Relationship, No sex	4	6.0
	Always	37	55.2
	Often	4	6.0
	Often-Sometimes (both responses given)	1	1.5
	Sometimes	7	10.4
	Never – Sexually active	4	6.0
	Missing/Undisclosed	1	

*HAART= Highly Active Antiretroviral Treatment

Immunological Data

Of the final participants, 66 had full immunological data (CD4, VL (absolute and log_{10} copies)) at interview (T2) time, 61 had full immunological data for artificial baseline (T1), 60 had CD4 prospective follow-up data and 62 had VL (absolute and log_{10} copies) prospective follow-up data at T3 (see table 6). Please see previous chapter for delineation between VL measures.

Table 6. Summary of immunological data, with mean, standard deviation and total number of respondents.

Cell Type & Data Collection Point		Mean	SD	*N*
$CD4^+$ (cells/mm^3)	T1: Retrospective data point	571.31	251.92	61
	T2: Baseline	567.64	263.29	66
	T3: Prospective data point	545.90	242.28	60
Viral Load (Absolute Copies)	T1	28461.67	90153.67	61
	T2	62015.48	3.39	66
	T3	39453.66	2.12	62
Viral Load (Log_{10} Copies)	T1	1.85	2.03	61
	T2	1.79	2.08	66
	T3	1.37	1.92	62

The mean values of $CD4^+$ T-lymphocyte cells suggests a sample of relatively healthy HIV^+ individuals, however there is a large variation in these values exhibited in the dataset. Viral load (VL) data also suggests broad variation, however this can be explained by the inclusion of participants both receiving and not receiving antiretroviral treatment. From mere inspection, one can see trends towards reduced $CD4^+$ levels over time. This represents a normal pattern of disease progression in these patients. VL data represents more variance, possibly due to the inclusion of a mixed-treated sample.

Tables of all zero-order associations between the dependent variable ($CD4^+$ T3) and all variables (continuous and categorical) conducted with two-tailed significance can be found in tables 7, 8 and 9. Tables 10 and 11 detail the one-tailed correlation between the HL measures and each immunological data collection point. Skewness and kurtosis in the independent and dependent variables can be found in Appendix C.

Table 7. Zero-order associations between the dependent variable ($CD4^+$ T3) and variables with continuous data.

Continuous Variable	$CD4^+$ T3		
	r	***p***	***N***
$CD4^+$ T1	.811*	<.01	59
$CD4^+$ T2	.797*	<.01	61
Age	.055	.676	61
Duration of HIV	.257	.054	57
MMSE	.157	.234	59
VL log10 T1	-.312*	.016	59
VL copies T1	-.238	.069	59
VL log10 T2	-.309*	.016	61
VL copies T2	-.232	.072	61
VL log10 T3	-.298*	.020	61
VL copies T3	-.241	.062	61
HADS – Anxiety	.078	.553	61
HADS - Depression	-.024	.853	61
Line Bisection Index of Left Lateralisation	-.118	.367	61
HPT Left Lateralisation Index Z Score (based on log transformation)	-.229	.095	54
Non-adherence Index (MMAS)	-.275	.071	44
Mean period between clinic visits	.213	.100	61
Number of sexual partners (prior 12 months)	-.027	.837	61
Condom use (12 months)	.066	.618	60
Alcohol use (prior 1 month)	.210	.104	61
Cigarette or Tobacco use (prior 1 month)	.205	.112	61
Marijuana use (prior 1 month)	.101	.436	61
Cocaine use (prior 1 month)	.118	.365	61

Table 8. Zero-order associations between the dependent variable ($CD4^+$ T3) and variables with categorical data with only two levels (employing *t* test)

Categorical Variable	***t***	***df***	***p***
Gender	.463	58	.645
Ethnicity	1.749	58	.086
Sexual Orientation	-.892	58	.376
HAART Medication	1.556	58	.125

Table 9. Zero-order associations between the dependent variable ($CD4^+$ T3) and variables with categorical data with more than two levels (employing ANOVA)

Categorical Variable	***F***	***df***	***p***
Education	.092	3, 58	.964
Employment Status	2.183	5, 59	.070
Relationship Status	.501	2, 58	.608
Mode of Contraction	.820	2, 59	.445
Condom Use	.210	6, 58	.972
Alcohol use (prior 1 month)	1.364	4, 59	.258
Cigarette or Tobacco use (prior 1 month)	.690	3, 59	.562
Marijuana use (prior 1 month)	.384	2, 59	.683
Cocaine use (prior 1 month)	.690	2, 59	.506

Table 10. Zero-order associations between the HPT and all $CD4^+$ T-cell data collection points.

HPT Sample		$CD4^+$ T1		$CD4^+$ T2		$CD4^+$ T3	
		r	*p*	*r*	*p*	*r*	*p*
Whole sample		-.130	.173	-.080	.273	-.054	.349
Ethnicity Split	European	-.147	.193	-.073	.330	-.150	.196
	African	-.006	.490	.091	.351	.389	.055
HAART Split	Yes	-.227	.075	-.138	.186	-.162	.156
	No	.240	.215	.057	.420	.402	.098

Table 11. Zero-order associations between the HPT and all $CD4^+$ T-cell data collection points.

LBT Sample		$CD4^+$ T1		$CD4^+$ T2		$CD4^+$ T3	
		r	*p*	*r*	*p*	*r*	*p*
Whole sample		-.017	.449	-.022	.429	-.086	.257
Ethnicity Split	European	-.037	.410	-.004	.490	-.108	.256
	African	-.025	.458	-.136	.269	-.154	.253
HAART Split	Yes	-.057	.357	.020	.447	-.114	.231
	No	.133	.305	-.087	.362	.023	.466

HEMISPHERIC LATERALISATION AND IMMUNITY IN HIV

First, simple bivariate correlations were conducted to assess the relationship between HL and the immunity outcome variables (at the prospective data collection point). A summary of the correlation coefficients and their relevant one-tailed probabilities are provided in table 12. It can be seen that the only significant relationship observed in this battery of tests is between the HPT and prospective VL copies. Whilst this relationship is encouraging, and is in the hypothesised direction, there were too many covariables with the VL data to adequately analyse this relationship further. This was, for the mostpart, because a mixed-treated sample (i.e. HAART-treated and HAART-naïve) was included, and therefore the effects of HAART on these outcome variables could not be appropriately controlled for. No further analyses for VL data were conducted.

Table 12. A summary of simple bivariate correlations (one-tailed) between left indices of the hemispheric lateralisation tests and outcome (prospective: T3) immunological and virological assessments.

HL Measure	$CD4^+$		$VL\ log_{10}$		VL copies	
	r	*p*	*r*	*p*	*r*	*p*
LBT	-.086	.257	.020	.439	.075	.282
HPT	-.054	.351	-.139	.158	-.241*	.038

In order to assess the impact of hemispheric lateralisation on disease progression in HIV, linear multiple regression (MR) analysis was applied. A MR analysis, with the prospective $CD4^+$ T-cell measurement as the dependent variable and retrospective $CD4^+$ T-cell measurement (artificial baseline), duration of HIV, mean time between clinic visits, non-adherence (using the Moriskey Medication Adherence Scale) and the log_{10} transformation of the Hemispheric Preference Test as predictors for the reduced whole sample (due to the errors of encoding for this variable) gave R^2=.76 (F change $_{(1,30)}$=.438, p=.513). This analysis indicated that the left index of the HPT contributed an additional, and non-significant, 0.3% of $CD4^+$ follow-up variance, beyond the contribution (76%) of the confounders.

CONFOUNDING VARIABLES

After completing the MR and finding no significant effect, it was decided further testing was required to assess confounding variables. Of those identified *a priori* from the literature only one proved to provide a significant relationship – baseline immunity. For outcome $CD4^+$ (T3), significant relationships were observed for HAART use ($t_{(51.36)}$= 2.23, p=.030); and baseline $CD4^+$ ($r_{(56)}$= .84, p<.001).

Due to the lack of significance of this main MR, further tests were run including the covariables identified statistically. A MR analysis, with the prospective $CD4^+$ T-cell measurement as the dependent variable and retrospective $CD4^+$ measurement (T1) and HAART medication, log_{10} transformation of the Hemispheric Preference Test (log_{10} HPT) and the left index of the Line Bisection Test (left LBT) as predictors for the reduced whole sample gave R^2=.73 (F change $_{(2, 46)}$=.785, p=.462). This indicates that the two HL tests contributed 0.7% of the variance observed in follow-up $CD4^+$ T-cell count beyond the contribution (71.6%) of the confounders, and were non-

significant. Table 13 summarises the original MR employing the covariables identified *a priori*, and the final MR employing both HL measures and the statistically identified covariables.

Table 13. A summary of the multiple regression (MR) analyses employed, their predictor and outcome variables, and covariables used. Outcome R^2 change and probability (p) value are provided. All MRs were conducted with the reduced whole sample.

Predictor Variable	**Covariables**	**Outcome Measure**	**R^2 change**	***p***
Log_{10} HPT – Index of LHL	Retrospective $CD4^+$ (T1) Duration of HIV Mean time between clinic visits Non-adherence to HAART medication	Prospective $CD4^+$	.003	.513
Log_{10} HPT LBT	Retrospective $CD4^+$ HAART medication	Prospective $CD4^+$	.009	.462

Due to the presence of skewness and possible outliers in the dataset, a nonparametric Spearman's *rho* correlation was conducted between the standardised prospective $CD4^+$ T-cell measurement ($CD4^+$ T3, residualised on $CD4^+$ T1 and HAART medication as covariates) and the log_{10} transformation of the Hemispheric Preference Test (log_{10} HPT). There was a statistically significant correlation between left lateralisation (left HPT) and standardised outcome $CD4^+$ T-cell count (*rho*=.234, p=.047, one tailed test, N=61).

MODERATING VARIABLES

Moderating variables were sought for further analysis of the dataset. To test the moderating relevance of these variables with other categorical data variables, chi-square (X^2) tests were conducted and t tests were conducted for continuous data variables. All analyses were conducted with two-tailed significance as they were unhypothesised assessments.

Ethnicity

An observed split in the data was ascertained between the two ethnicity groups (African and European). Descriptive statistics of the two sub-samples can be found in table 14.

Table 14. Descriptive statistics of the continuous data for the ethnicity-split sample, with mean and standard deviation values for each category.

Characteristic	Africans		Europeans	
	Mean	SD	Mean	SD
Age (years)	42.82	8.37	43.53	9.31
Duration of HIV (years)	6.22	5.30	6.36	6.90
MMSE (maximum range 25-30)	28.54	1.37	29.27	1.12
Mean period between clinic visits (days)	111.15	30.50	109.32	36.07
Non-Adherence (maximum range 0-4, 4 indicating maximum non-adherence)	.56	.89	.55	.72
Number of sexual partners (prior 12 months)	1.17	1.19	10.40	15.57
HADS Anxiety (maximum range 0-21)	6.91	4.32	7.37	4.44
HADS Depression (maximum range 0-21)	4.91	3.69	3.73	3.43
Line Bisection Index of Left Lateralisation (maximum range 0-10)	4.91	2.62	5.73	2.63
HPT Left Lateralisation Index Z Score (based on log transformation)	3.07	.05	3.04	.08
Follow-up (T3) $CD4^+$ T-cell Count	472.62	282.62	585.36	211.03

Significant differences were found between the two ethnicity groups for: gender ($x^2_{(1, N=68)}$=21.13, p<.001); level of education ($x^2_{(3, N=67)}$=13.15, p=.004); employment status ($x^2_{(5, N=68)}$=24.01, p<.001); sexual orientation ($x^2_{(1, N=68)}$=31.39, p<.001); mode of HIV contraction ($x^2_{(3, N=67)}$=13.24, p=.004); MMSE ($t_{(64)}$= 2.29, p=.025); and number of sexual partners in the prior 12 months ($t_{(45.01)}$=3.95, p<.001). A summary table of these significant categorical characteristics can be found in table 15. No significant difference was observed between outcome (T3) $CD4^+$ counts for these two groups ($t_{(58)}$= 1.75, p=.086).

The dataset was split by ethnicity: African (N=23) and European (N=45). Due to the reduced sample sizes Spearman's *rho* correlations were implemented. Spearman's *rho* correlations were conducted between the standardised prospective $CD4^+$ T-cell measurement ($CD4^+$ T3, residualised on $CD4^+$ T1 and HAART medication) and the $\log_{10}$ transformation of the Hemispheric Preference Test ($\log_{10}$ HPT) as predictor, split by ethnicity group. There were no statistically significant correlations between left lateralisation (left HPT) and standardised outcome $CD4^+$ T-cell count for the European sub-sample (*rho*=.241, p=.081, one tailed test, N=35) or for the African sub-sample (*rho*=.402, p=.055, one tailed test, N=17).

Table 15. Descriptive statistics of the categorical data for the ethnicity-split sample, with number of respondents and percentage of total respondents for each category.

Characteristic		Africans		Europeans	
		N	%	*N*	%
Gender	Male	9	39.1	41	91.1
	Female	14	60.9	4	8.9
Education	Primary	4	17.4	1	2.3
	Secondary	12	52.2	11	25.0
	Vocational	7	30.4	4	9.1
	Academic	0	0	28	63.6
Employment Status	Employed FT	6	26.1	35	77.8
	Employed PT	2	8.7	2	4.4
	Homemaker	1	4.3	0	0
	Unemployed (Health)	1	4.3	2	4.4
	Unemployed	13	56.5	4	8.9
	Retired	0	0	2	4.4
Sexual Orientation	Heterosexual	21	91.3	9	20.0
	Homosexual	2	8.7	36	80.0
Relationship Status	Single	11	47.8	22	50.0
	Married/Cohabiting	4	17.4	7	15.9
	In a Relationship	8	34.8	15	34.1
HAART Medication	HAART treated	16	69.6	32	71.1
	Untreated	7	30.4	13	28.9
Mode of HIV Contraction	Sexual Contact	10	43.5	33	75.0
	Injection Drug Use	0	0	1	2.3
	Medical Contact	5	21.7	0	0.0
	Unknown	8	34.8	10	22.7
Condom use (12 months)	Never – No relationship, No sex	5	21.7	5	11.4
	Never – Relationship, No sex	1	4.3	3	6.8
	Always	12	52.2	25	56.8
	Often	1	4.3	3	6.8
	Often-Sometimes (both responses given)	0		1	2.3
	Sometimes	3	13.0	4	9.1
	Never – Sexually active	1	4.3	3	6.8
	Missing/Undisclosed	0		1	

HAART

Additionally, the moderating variable of HAART medication was identified for testing. The inclusion of HAART-treated patients potentially confounded the results of the main analysis due to HAART's effects on general immunity in HIV patients. By analysing the implication of HAART as a moderator it would have been possible to ascertain whether including these patients corrupted the overall findings. Further, it was possible that there would be a medical significance of HL moderation of immunity in HIV patients either side of this clinically important milestone. After testing differences between the HAART-treated and HAART-untreated sub-samples,

very few significant differences were found between the groups in continuous or categorical variables. A significant difference was found between the two treatment groups for condom use ($x^2_{(1,\ N=68)}$=14.03, p=.029) and duration of HIV ($t_{(62)}$= 2.35, p=.022) only. A significant difference was also observed between treatment groups and outcome (T3) $CD4^+$ counts in these two groups ($t_{(50.80)}$= 2.06, p=.045). See tables 16 and 17 for group differences in continuous and categorical variables

Table 16. Descriptive statistics of the continuous data for the treatment-split sample, with mean and standard deviation values for each category.

Characteristic	**HAART Treated**		**Untreated**	
	Mean	SD	Mean	SD
Age (years)	43.83	8.93	42.00	9.07
Duration of HIV (years)	7.53	6.10	3.64	6.23
MMSE (maximum range 25-30)	28.91	1.28	29.31	1.15
Mean period between clinic visits (days)	114.29	34.01	99.5	32.40
Non-Adherence (maximum range 0-4, 4 indicating maximum non-adherence)	.55	.77	-	-
Number of sexual partners (prior 12 months)	5.54	8.68	11.45	20.47
HADS Anxiety (maximum range 0-21)	7.6	4.53	6.3	3.91
HADS Depression (maximum range 0-21)	4.29	3.73	3.75	3.09
Line Bisection Index of Left Lateralisation (maximum range 0-10)	5.29	2.44	5.85	3.08
HPT Left Lateralisation Index Z Score (based on log transformation)	3.05	.08	3.04	.07
Follow-up (T3) $CD4^+$ T-cell Count	574.90	265.94	466.13	137.45

Table 17. Descriptive statistics of the continuous data for the treatment-split sample, with number of respondents and percentage of total respondents for each category.

Characteristic		HAART Treated		Untreated	
		N	%	*N*	%
Gender	Male	35	72.9	15	75.0
	Female	13	27.1	5	25.0
Education	Primary	2	4.3	3	15.0
	Secondary	17	36.2	6	30.0
	Vocational	4	8.5	-	-
	Academic	24	51.1	11	55.0
Employment Status	Employed FT	25	52.1	16	80.0
	Employed PT	3	6.3	1	5.0
	Homemaker	1	2.1	-	-
	Unemployed (Health)	3	6.3	-	-
	Unemployed	14	29.2	3	15.0
	Retired	2	4.2	-	-
Sexual Orientation	Heterosexual	20	41.7	10	50.0
	Homosexual	28	58.3	10	50.0
Relationship Status	Single	22	46.8	11	55.0
	Married/Cohabiting	9	19.1	2	10.0
	In a Relationship	16	34.0	7	35.0
Ethnicity	European	32	66.7	13	65.0
	African	16	33.3	7	35.0
Mode of HIV Contraction	Sexual Contact	30	62.5	13	68.4
	Injection Drug Use	1	2.1	-	-
	Medical Contact	3	6.3	2	10.5
	Unknown	14	29.2	4	21.1
Condom use (12 months)	Never – No relationship, No sex	9	18.8	1	5.3
	Never – Relationship, No sex	4	8.3	-	-
	Always	24	50.0	13	68.4
	Often	4	8.3	-	-
	Often-Sometimes (both responses given)	1	2.1	-	-
	Sometimes	2	4.2	5	26.3
	Never – Sexually active	4	8.3	-	-
	Missing/Undisclosed	-	-	-	-

The dataset was split by HAART medication status: those taking HAART (*N*=48) and those not taking HAART (*N*=20). Spearman's *rho* correlations were conducted between the standardised prospective $CD4^+$ T-cell measurement ($CD4^+$ T3 residualised on $CD4^+$ T1) and the $\log_{10}$ transformation of the Hemispheric Preference Test ($\log_{10}$ HPT) as predictor split by treatment group. There was no statistically significant correlation between left lateralisation (left HPT) and standardised outcome $CD4^+$ T-cell count for the HAART treated sub-sample (*rho*=.153, *p*=.170, one tailed test, *N*=41).

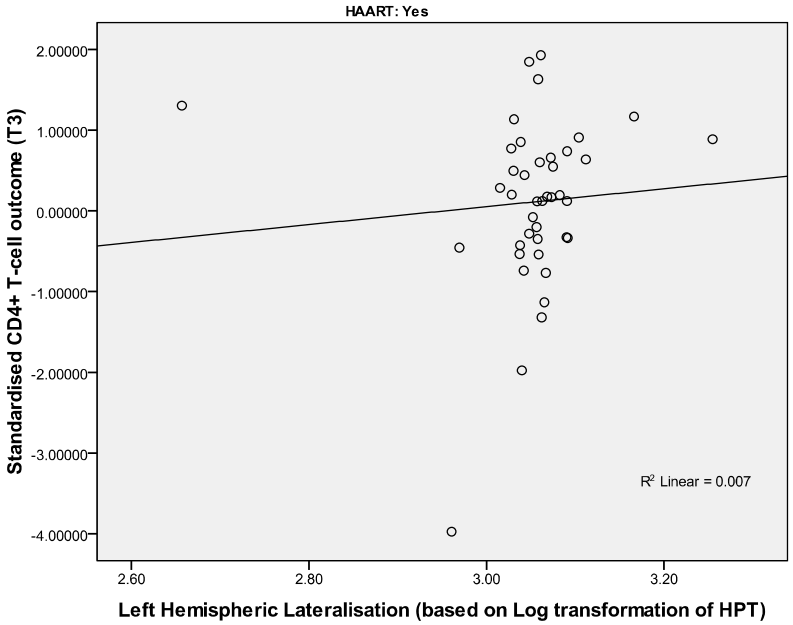


Figure 7. A scatterplot showing the correlation between left lateralisation (HPT $\log_{10}$ transformation) and standardised CD4+ T-cell follow-up for the HAART treated sub-sample.

There was a statistically significant correlation between left lateralisation (left HPT) and standardised outcome $CD4^+$ T-cell count for the untreated sub-sample (*rho*=.627, *p*=.019, one tailed test, *N*=11). Scatterplots of these correlations can be found in figures seven (HAART treated sub-sample) and eight (untreated sub-sample).

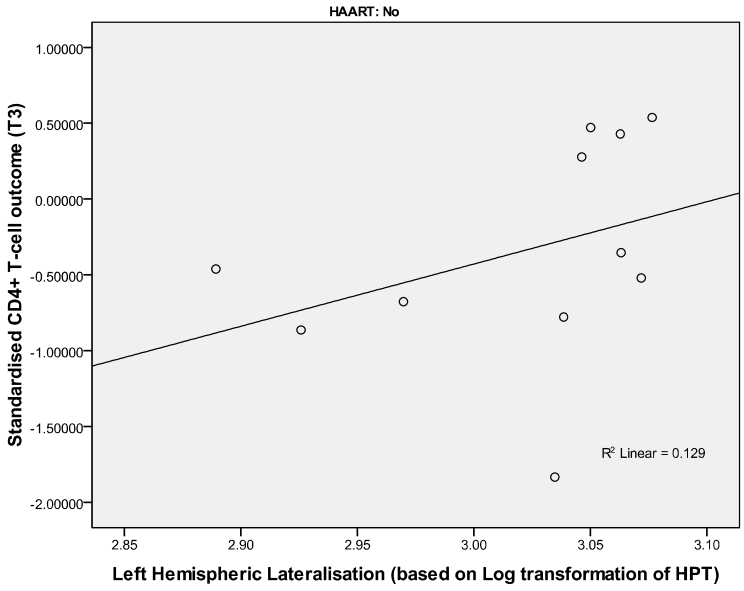


Figure 8. A scatterplot showing the correlation between left lateralisation (HPT $\log_{10}$ transformation) and standardised CD4+ T-cell follow-up for the untreated sub-sample.

POST-HOC EXPLORATORY ANALYSES – BEHAVIOUR

Due to the large volume of behavioural data taken we had a unique opportunity to examine the relation between HL and health behaviours. As these analyses were not hypothesised, only two-tailed bivariate correlations were employed to explore the possibility of LHL relating to the behavioural data collected in this HIV^+ sample. Both indices of LHL (the HPT and the line bisection task (LBT)) were used for this exploration, in both the full sample and the sub-samples split by ethnicity. The African sub-sample unanimously reported no cigarette/tobacco, marijuana or cocaine use.

Whole Sample

In the whole sample, LHL (LBT) was shown to have a significant relationship with self-reported marijuana use in the last month ($r_{(66)}$= -.26, p=.035). A trend was also identified in the whole sample, between LHL (LBT) and the number of sexual partners reported in the prior 12 months ($r_{(66)}$= -.22, p=.071). A summary of all correlations for the whole sample can be found in table 18.

Table 18. Summaries of the correlations for behavioural data in the whole sample. Correlation (r) and probability (p) values are provided. Those statistically significant correlations are denoted with *.

		Number of sexual partners (prior 12 months)	Mean period between clinic visits	Condom use (12 months)	Alcohol use (prior 1 month)	Cigarette or Tobacco use (prior 1 month)	Marijuana use (prior 1 month)	Cocaine use (prior 1 month)
HPT	*r*	.028	-.035	-.046	-.103	.030	-.015	.177
	p	.831	.796	.729	.430	.818	.911	.172
LBT	*r*	-.220	-.035	.132	.174	-.097	-.256*	.130
	p	.071	.784	.287	.157	.430	.035	.290

Split Sample

Europeans

In the split sample, two significant relationships were observed in the European sub-sample. A significant relationship was found between LHL (LBT) and number of

sexual partners in the past 12 months ($r_{(43)}$= -.36, p=.017; 2-tailed test); and LHL (LBT) and marijuana use in the past month ($r_{(43)}$= -.36, p=.016; 2-tailed test). Summaries of the correlations for behavioural data in the European subsample can be found in table 19.

Table 19. Summaries of the correlations for behavioural data in the European sub-sample. Correlation (r) and probability (p) values are provided. Those statistically significant correlations are denoted with *.

		Number of sexual partners (prior 12 months)	Mean period between clinic visits	Condom use (12 months)	Alcohol use (prior 1 month)	Cigarette or Tobacco use (prior 1 month)	Marijuana use (prior 1 month)	Cocaine use (prior 1 month)
HPT	*r*	.092	-.051	-.137	-.151	.128	.022	.252
	p	.568	.761	.400	.345	.427	.890	.112
LBT	*r*	-.356*	.115	.108	.024	-.211	-.357*	.120
	p	.017	.468	.487	.876	.163	.016	.432

Africans

In the African sub-sample, a trend was identified between LHL (LBT) and the mean time between clinic visits for HIV surveillance ($r_{(21)}$= -.36, p=.092; 2-tailed test). Summaries of the correlations for the behavioural data in the African subsample can be found in table 20.

Table 20. Summaries of the correlations for behavioural data in the African sub-sample. Correlation (r) and probability (p) values are provided. Those correlations with no data are denoted with -.

		Number of sexual partners (prior 12 months)	Mean period between clinic visits	Condom use (12 months)	Alcohol use (prior 1 month)	Cigarette or Tobacco use (prior 1 month)	Marijuana use (prior 1 month)	Cocaine use (prior 1 month)
HPT	*r*	.262	-.017	.349	.315	-	-	-
	p	.264	.942	.131	.176	-	-	-
LBT	*r*	.078	-.360	.148	.334	-	-	-
	p	.725	.092	.502	.119	-	-	-

Chapter 7

Discussion

THE EFFECTS OF HEMISPHERIC LATERALISATION ON PROGNOSIS IN HIV

The present study sought to examine the relationship between hemispheric lateralisation (HL) and prognosis in right-handed HIV-1^+ patients without a clinical diagnosis of AIDS. A recent review suggests that HL has a discernable relationship with immunity both in cross-sectional and prospective analyses, concluding that increased left HL is associated with better immune function (Sumner *et al.*, 2011). A prior study examining the effects of HL on immunity in HIV patients ($CD4^+$ and $CD8^+$ T-cells) showed that left HL predicted a better prognosis and right HL a poorer prognosis prospectively at 30 months (Gruzelier *et al.*, 1996). Despite the encouraging results from this earlier study, there was very little control for potential confounding variables, most notable of which was baseline immunity. It was the objective of the present study to replicate and advance these findings in a more diverse sample of HIV^+ patients, with more rigorous control for potential confounding variables, relating either to the disease, immunity or those neurological factors that may impact upon the assessment of HL. It was hypothesised that left HL would predict a better immune-specific prognosis in HIV, as indicated by a relatively higher prospective $CD4^+$ T-lymphocyte count. In order to assess this relationship, a number of potential covariables were also identified and analysed. These variables included those pertaining to potential psychosocial factors contributing to HIV prognosis (levels of depressive and anxious mood, relationship status, level of education and ethnicity), and those biomedical factors related to, or potentially causing factors related to, HIV prognosis (medication use and adherence, duration of illness, mode of contraction, condom use, frequency of condom use, addictive substance use and baseline immunity). Confounding variables for this relationship were selected *a priori* from indications from the literature, to be included in the main multiple regression analysis of the whole sample. The finding of this main multiple regression analysis examining the relationship between left HL and immunological outcome did not attain statistical significance. However, the dataset presented significant skewness (see Appendix C), with outliers being identified amongst the main independent variable of HL. Further analysis employing a simplified statistical strategy (Spearman's *rho* correlation), but retaining the objective of covariable control by way of using standardised residuals for outcome (T3) $CD4^+$ count, showed a significant, positive relationship between left

HL (HPT) and prospective $CD4^+$ T-cell count, independent of baseline $CD4^+$ level and HAART status. This analysis indicated that approximately 5.5% of the variance observed in outcome $CD4^+$ levels could be attributed to LHL, controlling for baseline $CD4^+$ counts and medication use, providing a small ($r \geq .1$) to medium ($r \geq .3$) effect size as designated by Cohen (1988). This finding, albeit relatively modest, is in line with those found in previous HIV^+ patient samples (Gruzelier *et al.*, 1996) and amongst the wider literature describing a relationship between left HL and better immunity (Sumner *et al.*, 2011).

Moderation Effects

Ethnicity

A potential moderating factor was identified in ethnicity, by the observation of potential relationships between this variable and several other confounding variables (e.g. gender, level of education, employment status, sexual orientation, mode of HIV contraction, cognitive function and number of sexual partners). Statistical analyses of these potential confounding variables showed significant relationships between $CD4^+$ T-cells, the artificial baseline (retrospective) $CD4^+$ count and HIV medication status (Highly Active Antiretroviral Treatment; HAART).

As the sample sizes were reduced for the moderator analyses, Spearman's *rho* non-parametric correlations were employed. No significant associations were observed in the European or African sub-samples, however both correlations were near statistical significance.

These findings suggest that ethnicity may be a moderating factor in the HL-HIV progression relationship; however the data presented within this current sample were not of a sufficient size to adequately ascertain the extent of this moderation. The rationale behind ethnicity as a moderating factor has merit when considering the distribution of viral subtypes of HIV, the associations of HAART efficacy and viral virulence within these different subtypes. Subtype B is more commonly found in Europeans than other viral subtypes (Klimas *et al.*, 2008; Roquebert *et al.*, 2009). Africa has regional subtype prevalences, with the continent as a whole showing predominance of subtypes A, C, D and CRF02_AG (Klimas *et al.*, 2008; Roquebert *et al.*, 2009). It has been suggested that of all the main global HIV viral subtypes, clade B (most prevalent in Western Europe) presents a slower progression of HIV disease

than all other non-B subtypes (Spira *et al.*, 2003). Additionally, the chemical agents in the spectrum of HAART have largely been designed for, and tested with, subtype B virus – making their efficacy most pertinent to those patients with this viral subtype (Spira *et al.*, 2003) – mostly Western nations (Western Europe, North America and Australia). It is therefore possible that both ethnicity and HAART together may mask or moderate the HL-HIV prognosis relation. Thus, the examination of HAART as a moderator was conducted as well, however the sample was too small to test both factors as moderators together.

Medication

The examination of HAART medication as a potential moderating factor has clinical significance and practical value amongst the present dataset. The study was initially intended to only examine HL effects in HAART-naïve patients, and so examining the implication of including HAART-treated patients was a valid means of understanding this methodological compromise. Further, as the initiation of HAART treatment signifies a clinically distinct period in HIV disease (initiated once $CD4^+$ T-cell levels decline below 350/mm^3 (Hammer *et al.*, 2006)), whether the HL-immune relationship differs in either side of this point can help to understand the biological mechanisms of the relationship and the practical application of targeted interventions.

The Spearman's *rho* strategy using standardised residuals to account for the confounding variable of baseline (T1) $CD4^+$ count provided a significant, positive relationship between left HL (HPT) and prospective immunity only in those patients who were not receiving antiretroviral treatment. This analysis indicated that approximately 39.3% of the variance observed in outcome $CD4^+$ levels could be attributed to LHL, controlling for baseline $CD4^+$ counts, providing a large ($r \geq .5$) effect size as designated by Cohen (1988).

These new findings present a new dimension to the current knowledge on HL in HIV disease. The Gruzelier group study (1996) included patients from an early-HIV Zidovudine drug trial, with 11 patients taking a placebo and 16 drug-treated patients, so the present findings are not entirely concordant. However, modern HAART comprises many new drug classes, being prescribed as combinations in either fixed doses or cocktails, therefore complicating the direct comparison from the Gruzelier study to the present findings. Whilst it was beyond the remit of the present study, it is

possible that there may be further differences amongst types of treatments within the HAART-treated group, with chemical classes being used to combat various different parts of the HIV virion life-cycle, from cell infiltration to reverse-transcription. It is also possible that the effects of HL on HIV progression in the Gruzelier study were only observed in those patients taking the placebo, and that this relationship (as it was measured over 36 months, and in a proportional placebo-to-treatment sample split (*N*=11:16)) was strong enough to withstand a null relationship in the treated sub-sample, however this kind of testing was not reported. This theory is not entirely speculative, as it is how the findings appear in the present study, with overall statistical significance in the whole sample employing the same statistical test as that which described discrepant findings amongst treatment groups, in more unevenly split groups (non-treated versus HAART treated, *N*=11:41).

Across the broader context of the literature, these findings support those of other studies that have examined the effects of HL on T-cells in patient groups (Ivashkova *et al.*, 2002; Meador *et al.*, 2004; Tarkowski *et al.*, 1995; 1998). Further, the effect size observed (*r*=.627) was of similar magnitude (i.e. "large" in Cohen's designation (1988)) to that reported as a mean effect size (*r*=.536) amongst the HL-immunity literature (Sumner *et al.*, 2011); despite the present study's higher degree of third variable control. However it should be noted that this sub-sample size (*N*=11) was significantly lower than the mean for the previously reviewed studies (*N* mean=39.72) and smaller sample sizes can, by their nature, produce larger effect sizes (Givens *et al.*, 1997). The lack of statistical significance for the relationship in the HAART-treated sub-sample is in contravention to the literature, yet when considering the statistical significance of the whole group this finding may not be erroneous; however the findings will require replication.

Differential predictive value of LHL in prospective immunity for non-treated HIV patients

Why left HL is predictive of $CD4^+$ outcome in treatment-naïve patients, but not HAART-treated patients, is not immediately clear. It is interesting that the non-significance of the HAART-treated sample correlation is attenuated by the relationship in HAART-naïve patients when the sample is analysed as a whole. This is further complicated by the consideration of the mixed-treatment sample (albeit with a

less sophisticated monotherapy based treatment) in the Gruzelier group study (1996). It is possible that as HAART has a directly anti-viral action, with an indirect immunological action, and HL has an immunological action, with potentially indirect antiviral action, their presences may compete with each other in the overall outcome on HIV (see figure 9) or they may possibly interact with each other. As HAART controls HIV viraemia, the circulating numbers of immune cells (most notably T-cells) are increased (Deeks, 2006; Simon *et al.*, 2006). HL is suggested to modulate immunity at the system and cellular level (Koch *et al.*, 2006; Tarkowski *et al.*, 1995; 1998), which could, in turn, help to control HIV viraemia. Equally, it could be possible that if both left HL and HAART are working in unison toward immunopotentiation, that the effects of HL in the presence of HAART may be nullified or masked.

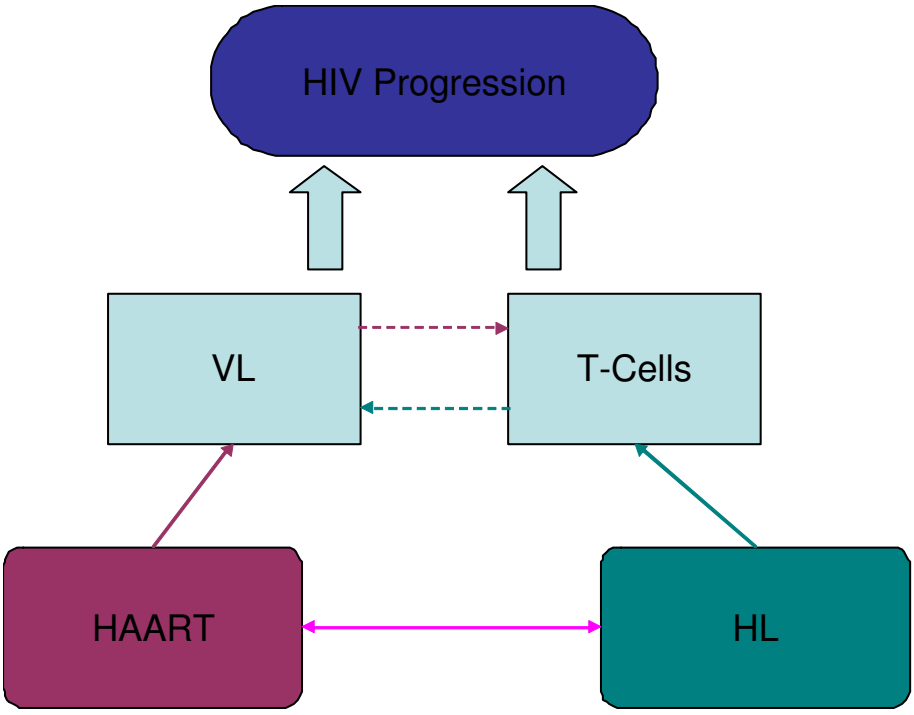


Figure 9. A proposed conflict of influence of HL and HAART in HIV prognosis. Both indirect and direct influences of HL and HAART can be possible on both T-cells and VL.

When examined within the context of the broader literature, it is clear that HAART affects other aspects of immunology that are affected by HL. For example, induction of HAART treatment has been shown to affect plasma levels of interleukin (IL) -10,

neutrophil function and antigen-induced lymphocyte proliferation (Amirayan-Chevillard *et al.*, 2000; Lange *et al.*, 2002; Mastroianni *et al.*, 1999; Powderly *et al.*, 1998). When compared to HIV^- controls, and untreated HIV^+ patients, the elevated levels of plasma IL-10 observed in HIV disease have been described to return to levels comparable with healthy controls (Amirayan-Chevillard *et al.*, 2000, Orsilles *et al.*, 2006). Dziedzic *et al.* (2003) demonstrated that left-sided haemorrhagic stroke induced increases in circulating IL-10, suggesting that left HL effects on this anti-inflammatory cytokine may be masked by HAART treatment, as they effectively both work to decrease circulating IL-10. Similarly, impaired lymphocyte proliferation to antigen observed in HIV disease has been seen to rebound after initiation of HAART, to levels analogous to healthy controls (Lange *et al.*, 2002; Powderly *et al.*, 1998), which has been associated with an increase in memory T-cells (Wendland *et al.*, 1999). Kang *et al.* (1991) and Ivashkova *et al.* (2002) both reported that left HL (in higher prefrontal EEG activation and right-hemispheric stroke respectively) was related to higher levels of lymphocyte proliferation to a T-cell specific mitogen (Phytohaemagglutinin), indicating that again HAART may mask left HL effects, as they both work in the same direction: to increase lymphocyte proliferation. The Ivashkova group (2002) also reported an increase in the function of neutrophils following right-sided stroke, with Koch *et al.* (2006) additionally reporting an increase in white blood cell count after stroke in the same hemisphere, these findings were inverse for stroke in the left-hemisphere in both studies. HAART has been demonstrated to increase the functional activity of neutrophils and monocytes in HIV patients, who classically exhibit a detriment in this index of immunity (Mastroianni *et al.*, 1999).

However, on examination of other immune indices demonstrated to be affected by HL, the pattern of HL and HAART working to influence immunity in the same direction does not consistently hold. The Koch group (2006) examined C-reactive protein (CRP) in patients after either left- or right-sided ischemic stroke. In this study they found that when the two groups were compared to each other, right-sided ischemic patients showed relatively lower levels of CRP than left-sided stroke patients (Koch *et al.*, 2006). This marker of inflammation has been shown to be elevated in HIV patients, and this elevation is not ameliorated by the use of HAART (Regidor *et al.*, 2011). Whilst CRP may be naturally elevated in a stroke patient, irrespective of location of ischemia, there is a differential observed from lateral

localisation that is not apparently mimicked by HAART in HIV. Similarly, natural killer cell activity (NKCA) has been demonstrated to be affected by HL, with the Kang (1991) and Davidson (1999) groups describing increases in NKCA in left-lateralised participants, and Kang *et al.* also describing an inverse relationship for right-lateralised participants. In HIV disease, NKCA is markedly increased compared to healthy controls, and this increase normalises with HAART (Parato *et al.*, 2002). Therefore, HAART does not seem to echo the actions of left-HL across all immune parameters thus far mutually researched. It is possible that the left hemisphere has more than just an immunopotentiating role. Given that these elevated levels of CRP and NKCA are above healthy parameters in HIV, there would be no need to increase them. Speculatively, it could be possible that the left hemisphere also serves as an allostatic decision maker, whereby it only acts to increase immunity if that increase is required.

Whilst it was beyond the remit of the present study to evaluate other indices of immunity in this HIV sample, immune cells do not work in mutual exclusion, and so analysis of HL in relation to these other aspects of immunity would be beneficial. Not only would this help to understand why the presence of HAART may obliterate HL effects on HIV-specific immunity, it could also potentially serve to describe the mechanisms of how left HL provides a beneficial prognosis in untreated HIV disease. The chemical agents of HAART, whilst effective and crucial to prolonged survival with an HIV diagnosis, are nonetheless beleaguered with tolerance and toxicity effects and can cause potentially severe side-effects in patients (Deeks, 2006). The longer a patient can maintain a good standard of health before having to commence HAART treatment, the longer these negative consequences can be postponed, thereby increasing quality of life and potentially survival. Of equal merit is the consideration that if left-HL provides a better pre-treatment HIV prognosis, then this could become a potential target for intervention; crucially an intervention without financial burden, restriction of freedom (e.g. avoiding drug interactions, maintaining a strict pill regimen) or the necessity of further clinic attendance or medical monitoring. An interesting avenue of future research would be to examine the effects of activating left-HL in HAART-naïve HIV patients across multiple indices of immune activity and function.

Mediation Effect – The lack of contribution of mood

The present study showed a lack of implication of mood in mediating the observed relationship between HL and HIV prognosis. In the present participant sample, neither indices of mood (anxiety and depression as measured by the Hospital Anxiety and Depression Scale; HADS) correlated significantly with HL or with outcome $CD4^+$ T-cell count. Previously, Gruzelier *et al.* found that mood impairment (measured by the HADS and the Profile of Mood States) predicted outcome helper T-cell counts in their sample, although did not correlate cross-sectionally at study commencement. Unfortunately the Gruzelier group did not report including mood in their HL-immune relation tests, so it is not possible to make a direct comparison here. Anxiety and depression are both known to have deleterious effects on HIV progression, in subjective experience and clinical prognostic markers (Chida & Vedhara, 2009; Hartzell *et al.*, 2008; Leserman, 2003). Equally, mood has implications on HL, with overall activation being suggested to switch from left to right during certain adverse mood states or situations such as exam stress (Lewis *et al.*, 2007). Furthermore, there are suggestions that prolonged states of stress may result in volumetric reductions in the left medial prefrontal cortex (Cerqueira *et al.*, 2008), potentially physically altering the activational asymmetry of this region.

Although it might be expected that there should be a mediation effect here, due to the well-documented relationship between mood and HIV prognosis, the present findings are not discrepant with the formerly reviewed HL-immunity studies. Of those studies that also examined mood alongside HL-immune assessments, there was a consensus that the observed HL-immune relationships were independent of mood or stress (Davidson *et al.*, 1999; Kang *et al.*, 1991; Meador *et al.*, 2004). In fact, one of the studies examining prefrontal activation asymmetry found no significant differences in mood between left and right HL groups (Kang *et al.*, 1991).

In a range score of zero to 21 in both scales, the present sample attained a mean score of 7.32 (standard deviation= ±4.42) in the anxiety subscale and 4.15 (standard deviation= ±3.52) in the depression subscale. Although no concrete cut-off scores for indicative diagnosis have been set, the originators Zigmond and Snaith have suggested that a score of between seven and eight on either scale would indicate "possible" morbidity, and a minimum score of 10 or 11 indicates "probable" morbidity (Herrmann, 1997). With these guidelines, the present sample would

indicate a mean level of "possible" anxiety, and a sample mean below "possible" depression. In comparison to the sample from the Gruzelier group's (1996) study, the present sample represented a more anxious and more depressed mean score; however the scores, and variance thereof, were comparable to others observed in HIV populations (Savard *et al.*, 1998) and healthy populations (Crawford *et al.*, 2001).

HEMISPHERIC LATERALISATION & HIV-RELEVANT BEHAVIOUR

Exploratory analyses were conducted to assess the implication of HL on behaviour relevant to HIV health. These analyses were conducted in order to understand potential behavioural mechanisms involved in the HL-immunity relationship within the HIV context. A number of significant associations were uncovered, as well as some potential trends amongst the whole sample, and ethnicity-split subsamples.

High-Risk Sexual Behaviour & HL

Number of Sexual Partners

Of most relevance to HIV, a negative relationship trend was uncovered between LHL (as measured by the LBT) and the number of sexual partners reported in the prior 12 months in both the whole sample and European subsample. In the whole sample the effect size of the relationship was between small (0.1) and medium (0.3) as designated by Cohen (1988), and was non-significant (p=.071); which once reduced to the European subsample increased to over a medium effect size and attained statistical significance (p=.017). This relationship suggests that left HL is associated with fewer sexual partners, most specifically in Europeans, and this is the first documented association of this sort in the contemporary literature on HL and health, to the best of my knowledge.

This finding is important because of the associated risks for accelerated HIV progression following superinfection with more than one type of HIV virus (Blackard, *et al.*, 2002; Smith *et al.*, 2005), often associated with having multiple partners. It is also a high-risk behaviour for an individual to contract another sexually transmitted infection or blood-borne pathogen, such as Syphilis or Hepatitis, which has consequences not only for accelerated pathogenicity, but also for increased HIV transmission probability (Gore-Felton & Coopman, 2008; Kaul, *et al.*, 2008; Soriano *et al.*, 2010). Clearly more sexual partners do not necessarily suggest superinfection,

however the risk of superinfection is obviously higher for an individual who has many sexual partners in comparison to an individual who has none.

There is currently no data on the impact of HL on high-risk behaviours such as the number of sexual partners, with or without implications of ethnicity. As it would be classified as approach behaviour, associated with left-HL (Coan & Allen, 2003; Davidson, 2003), it is curious that the relationship is not in the opposite direction. Additionally, risky behaviour is largely a left-brain cognitive process (Krain *et al.*, 2006), which one would again expect to result in an opposite correlation. It is possible that, as the left hemisphere is responsible for analytic processing (Bradshaw & Nettleton, 1981), this type of thinking may supersede approach, impulsive or risk-taking behaviour.

It should be noted that no such associations were observed for condom use in the whole sample or European subsample, which has a significant implication for this risk behaviour. Were there a similar association between HL and condom use, the implications of the present finding would be extremely clear. Furthermore, there was no observable relationship between number of sexual partners and condom use either.

Condom Use

The collection of condom use data was a synthesis of condom use surveillance weighed against sexual activity. The scoring of the scale was proportional to the risk of the behaviour (i.e. a score of 1 represented low risk sexual behaviour, a score of 6 the highest risk sexual behaviour).

In the African subsample a trend was observed for an inverse relation between LHL (as measured by the HPT) and condom use. Whilst this was only an observed trend, and the correlation did not attain statistical significance, the effect size was medium in accordance to Cohen's (1988) guidelines. Sample size calculation, given the correlation coefficient, an alpha level of 0.05 and a beta level of 0.8 projected that 61 participants would be required to attain a statistically significant result. However, the current sample consisted of just 23 African patients.

Whilst it should be noted that the analyses for the whole sample and subsamples were not significant, it is worthwhile considering why these discrepant findings were observed in the present dataset. As condom use is relevant not only to the disease progression of the individual, but also to the development of the global pandemic of HIV (by the facilitation of transmission), it is an important factor to consider in HIV

behavioural medicine. Further studies need to investigate more clearly the HL-condom-use relationship.

Many variables have been uncovered as impacting upon condom use in a recent review by Sarkar (2008). This review details religion, ethnicity, sexuality, gender and social barriers to condom use; suggesting in synthesis that African women are one of the most vulnerable groups for condom non-use (Sarkar, 2008). The barriers presented to this demographic group come from societal conventions concerning modesty, submissiveness and the association of condoms with promiscuity; religious barriers (most specifically within Catholic communities) and social equality, whereby women have less opportunity or right to negotiate condom use (Sarkar, 2008).

Addictive Substance Use & HL

Amongst both the full sample and European subsamples, a negative association was observed between LHL (as measured by the LBT) and reported marijuana use in the prior month to interview. Amongst the whole sample this relationship constituted a small to medium effect size, which then grows to a medium effect size in the European sample (Cohen, 1988). It is worthwhile noting at this stage that no African patients in the present sample reported using marijuana in the past month, hence the lack of association among them. Similarly there were no reports of cigarette/tobacco or cocaine use amongst that subsample. In the European subsample, further relationship trends were uncovered between LHL (LBT) and cigarette/tobacco use (negative association), and LHL (HPT) and cocaine use (positive association) in the past month.

Interestingly, the associations observed for the two stimulants (nicotine, cocaine) are divergent, with the cigarette/tobacco trend showing negative directionality and the cocaine trend showing positive directionality. Clearly further research in to these phenomena is required, both within and without the HIV context.

HIV Clinic Attendance & HL

Although not statistically significant, a trend was observed amongst the African subsample for the mean time between HIV clinic visits. This association (p=.092) provided a negative relationship between LHL and mean time between visits. This suggests that in the African subsample, LHL was predictive of smaller time gaps between visits to the clinic for HIV surveillance. Clinic attendance is important for

HIV surveillance, both in terms of the immunological assessments that are required, but also to assess the suitability and efficacy of HAART regimens. Previous studies have suggested that ethnic minority status predicts greater non-attendance to HIV clinics, amongst other variables (Catz, *et al.*, 1999; Israelski *et al.*, 2001), which could explain why this relationship was only found in the African subsample. The European subsample showed a mean time between visits of 109.32 days (standard deviation ±36.08 days) and the African subsample showed a mean time of 111.15 (standard deviation ±30.49 days), differences between these means were not significant. Given that minorities may visit clinics less or manifest longer time gaps between visits, identifying those with low left-HL could possibly enable the identification in advance of those who may be at risk for poorer clinic attendance, and thus would require more reminders.

It is possible that HL is associated with clinic attendance in the present subsample due to its associated effects on behaviour – with LHL being associated with approach behaviour (Davidson, 2003). However, it should be noted that less clinic attendance is associated with other factors, not just ethnicity. In fact, younger age, less severe illness, heterosexual orientation and lower perceived social support have also all been correlated with non-attendance to HIV clinics (Catz *et al.*, 1999; Israelski *et al.*, 2001). In this regard, the present African sub-sample was comprised of more women, and more heterosexuals than the European sub-sample, however the duration of illness (a possible indicator of illness severity) and age were comparable between the sub-groups.

THE ROLE OF HEMISPHERIC LATERALISATION IN IMMUNITY AND HIV-1 PROGNOSIS

The synthesised literature review presented in chapter 3 and published (Sumner *et al.*, 2011) concerning HL and immunity provides compelling evidence for differential immunomodulation by the two hemispheres of the brain. All of the presently reviewed studies describe an immunopotentiating role of the left hemisphere and an immunosuppressant role of the right. The combined weight of the reviewed studies is such that the research focus must now shift from hypothesis testing to exerting more control in order to understand the depth and mechanistic dynamics of the relationship. Indeed, the next logical step in the field is to continue these examinations exerting more methodological control over potential confounding variables, and the

examination of moderating and mediating factors. One already nascent avenue of such research is the examination of specific areas of the brain that may drive the communication of immunomodulation to the periphery, and the associated chemical transmitters that provide this communication. The findings of the systematic review now suggest the discipline of HL-immunity research can be considered to be evidence-based, and must now expand to consider the broader context of health and disease consequences; such as the assessment of HL's prognostic capability amongst other leading world health concerns (e.g. cardiovascular disease, cancer), vaccine efficacy and intervention strategy.

One of the previously reviewed studies suggested that HL could explain some of the variance observed in HIV pathogenesis (Gruzelier *et al.*, 1996). HIV pathogenesis is known to vary greatly between individuals, and whilst some risk factors for this variance are known, much of the variance observed is still unexplained (Lama & Planelles, 2007; Langford *et al.*, 2007; Leserman, 2003). In the spirit of the future research indications of the systematic review conducted, the present study sought to advance those findings in a contemporary sample of HIV patients, while exerting more control over confounding variables than Gruzelier *et al.* (1996) to assess whether these findings would withstand more rigourous testing. The present study provides some support for HL influencing HIV progression, and furthers this support to describe a moderator - HIV medication. However the findings are far from resolute, and will require replication and further advancement to incorporate more comprehensive immunological analyses in order to stand amongst the existing literature.

In line with the synthesised findings of the wider literature, the present analyses support the argument of an immunopotentiating role of the left hemisphere. In accordance to the studies of Ivashkova *et al.* (2002), via transcranial electromagnetic stimulation to the ischemic left hemisphere, Gruzelier *et al.* (1996), via prospective association, and Meador *et al.* (2004), via post right-hemispheric surgery prospective association, the current analyses suggest left HL predicts upregulated $CD4^+$ T-cell populations. Critically, the present research has extended these previous findings by exerting more methodological control over potential confounding variables associated with both HIV-immunity and laterality, and including a broader (perhaps more representative) profile of HIV^+ patients, all of which the former study did not do. However these findings do need to be viewed with a degree of caution. Crucially, not

all analyses provided significant results for the whole sample, or moderator-analysed sub-samples. The planned multiple regression analysis yielded no significant results, but the more simple correlational analysis did, for both the full sample relationship and the HAART-moderated sub-sample analysis, but not for the ethnicity-moderated analysis. It is possible that the large amount of skewness present in the main independent variable is responsible for this discrepancy, and therefore that there was a critical methodological flaw in the research design – namely the measures of HL employed. Of the previously reviewed studies that required HL assessment (i.e. HL was not created by cerebral trauma), all of the studies used EEG assessments with or without accompanying neuropsychological evaluation.

Neurobiological Pathways for HL Driven Immunomodulation

Although specific exploration of the potential neuroimmune pathways that facilitate this relationship was beyond the remit of the present work, there still exists literature which could elucidate the relationship between HL and immunity. The main findings of the studies relating HL to immune function are further elaborated when including research that has looked into specific areas of the brain and immune function. One of the previously reviewed studies already took that step. The Davidson research group related their findings not just to HL patterns but also to the areas of HL-brain activity, highlighted by EEG, most strongly related to peripheral immunity. This study revealed that the HL-differences at mid-sensorimotor and anterior temporal regions were the differences most strongly related to natural killer cell activity (NKCA) (Davidson *et al.*, 1999). These results have been echoed in other studies using positron-emission tomography (PET) in various samples of participants, with the addition of more specific areas that are discoverable with such methodology (Lekander *et al.*, 2000; Matsunaga *et al.*, 2008; Ohira *et al.*, 2008; Tashiro *et al.*, 2001; Wik *et al.*, 1998). These studies have also found that greater immune function (NKCA and lymphocyte proliferation) is related to activity in the secondary visual, motor and sensory cortices; hippocampus; putamen; thalamus; anterior cingulate cortex; posterior parietal cortex; and several areas of the prefrontal cortex (Lekander *et al.*, 2000; Matsunaga *et al.*, 2008; Ohira *et al.*, 2008; Tashiro *et al.*, 2001; Wik *et al.*, 1998).

One PET study by Matsunaga *et al.* (2008) examined the relationship between areas of cerebral activation and immune indices in positive and neutral affect. They found a positive correlation between NKCA and dopamine (DA). The increases in NKCA and DA were related to increased activity in areas of the brain such as the medial prefrontal cortex, thalamus, hypothalamus, cerebellum, posterior cingulate cortex, subcallosal gyrus and superior temporal gyrus during experience of positive affect (Matsunaga *et al.*, 2008). This echoes the findings of the Davidson work group who demonstrated, also under conditions of positive affect, that regions in the mid-frontal brain (medial prefrontal gyrus) and mid-temporal region (medial temporal gyrus) on the left side of the brain correlated with NKCA (Davidson *et al.*, 1999). DA receptors have also been described on T-cells, B-cells, granulocytes and monocytes/macrophages as well as NK cells (Basu & Dasgupta, 2000; Levite, 2008; Wrona, 2006). Studies using mouse models have also found lateralised effects on T-lymphocyte proliferation and NKCA after unilateral lesions to dopaminergic brain regions such as the nucleus accumbens and striatum, with left-sided lesions decreasing NKCA (Deleplanque *et al.*, 1994). In synthesis, this suggests that left-sided dopaminergic areas of the brain are related to higher levels of NKCA, meaning DA could serve as a neurotransmitter in brain-to-immune communication.

Further, associations have been made between dopaminergic areas of the brain, such as the mesostriatum and substantia nigra, and the production of Substance P (SP) in rats and humans (Gerfen *et al.*, 1991; Mai *et al.*, 1986). This could potentially impact upon the HL-HIV immunity relationship with relevance to the previously discussed implications of SP in HIV. To recapitulate, SP has a reciprocal relationship with HIV virus *in vivo*, creating a feed-forward cycle where the existence of one upregulates the other (Ho *et al.*, 2002; Kopnisky *et al.*, 2004; Leserman, 2003). The presence of SP stimulates the release of proinflammatory cytokines such as Tumour Necrosis Factor (TNF), which promote the release of new target cells for infection (Ho & Douglas, 2004; Kopnisky *et al.*, 2004; Li *et al.*, 2001). Moreover, SP promotes the activation of CCR5 on target immune cells – a particularly relevant factor for R5-tropic HIV virus (Li *et al.*, 2001), and can activate cells latently infected with HIV (Ho & Douglas, 2004; Li *et al.*, 2001). HIV virus also increases the blood levels of SP, thereby creating this positive feedback cycle (Ho *et al.*, 2002). Therefore, if dopaminergic regions of the brain can impact upon the generation of SP, which can then in turn

impact the availability of HIV target cells, there is the possibility that DA and SP may have a synergistic impact upon HIV virology and pathogenesis. However, the directionality of the relationship between DA and HIV is not altogether clear, given its relationship to SP, and so this postulation requires deeper investigation.

Another potential means of explaining the mechanisms behind the HL-immunity relationship is via the autonomic nervous system (ANS). The cholinergic anti-inflammatory pathway is a well described interface between the brain and the immune system, and relies on the parasympathetic cascade of acetylcholine to inhibit the proinflammatory response originating in monocytes (Tracey, 2002). More well-documented is the relationship between the sympathetic division of the ANS and immunity. The sympathetic nervous system (SNS) innervates primary and secondary lymphoid organs (Banks, 2004; Wrona, 2006). There are also receptors for β-adrenergic (sympathetic) agents present on the surface of a wide range of immune system cells (Besedovsky & Del Ray, 1996; 2007; Taub, 2008). Recent evidence has suggested an immunomodulating effect of the SNS on $CD8^+$ T-lymphocytes (Grebe *et al.*, 2009). Further, an experiment involving the surgical sympathectomy of mice abolished the HL-immune relationship when the surgery was conducted at the T1 vertebra (removing SNS activity), however sectioning at lower vertebrae did not have these effects (Moshel *et al.*, 2005). It is therefore possible that the SNS has mediating effects in the HL-immune relationship.

LIMITATIONS

Whilst the present study was designed to replicate and extend the findings of the Gruzelier group using more meticulous methodology and strict control over third variables, there exist many limitations in this study as well. As an overall comment on limitation, the extensive statistical testing risked a type I error, it was however necessary given the initial lack of significance, and general paucity of research in this specific field.

Sample Size & Composition

Perhaps the most vital limitation to the present study is the sample size, further compounded by the previously discussed issues with encoding of the HPT. The initial

statistical projection suggested a sample of between 67 and 77 participants in order to adequately assess the relationship using multiple regression analysis. A total sample size of 68 was finally attained, after exclusion of three participants whose data could not be included due to comprehension problems. This sample was then further reduced to 63 for all analyses employing the HPT as a variable (due to encoding errors), bringing the total to just below that which was required. Furthermore, several patients' immunological data were not available for all three data collection points, meaning those analyses that involved immunological parameters were further reduced. Whilst it is not being suggested that this issue was the only one responsible for the lack of statistical significance in the main analysis, it is a fundamental issue in statistical analyses, and as such, cannot be overlooked as having a potential impact.

One of the main reasons for the difficulty in attaining an adequate sample was perhaps the amount of exclusion factors that were ascertained before recruitment. For example, by excluding substance-dependent patients the potential available sample could have been significantly reduced. It has been supposed that injection drug users constitute a significant proportion of HIV^+ patients in Western samples alongside men who have sex with men (MSM) (UNAIDS, 2010). Moreover, there is a high prevalence of addictive-substance dependence amongst HIV samples outside of the injection drug use domain (Cabral, 2006; Fiellin, 2004). It was important to exclude all such cases in the present study due to the associated impact that addictive-substance dependence has on both HIV progression, HIV medication adherence and cerebral function (Basso & Bornstein, 2000; Jernigan *et al.*, 2005; Uldall *et al.*, 2004). Similarly, the exclusion of patients with a clinical diagnosis of psychological morbidity may also have significantly reduced the potential sample size. There is a well-documented high prevalence of psychological comorbidity within HIV^+ patients (Gibbie *et al.*, 2006; McCain *et al.*, 2003; Milam, 2006), with further well-documented consequences for such comorbidities upon disease progression (Chida & Vedhara, 2009; Evans *et al.*, 2002; Klis *et al.*, 2011). Whilst these exclusion factors are considered to be one of the key strengths of the present study as they reflect methodological control, they could also have led to limiting the potential sample size and/or diversity.

The composition of the present sample is also an area for concern, and impacts upon the generalisability of the present findings. With the previously discussed issues of the high prevalence of substance dependence and psychological comorbidity within HIV patient samples, the present sample has limited generalisability to general HIV populations. Furthermore, although the present sample exhibited some ethnic diversity, the two subsamples of Europeans and Africans were neither equal in number nor composition. Moreover, as the sample was composed of mainly Africans and Europeans, the representation of various HIV viral subtypes will undoubtedly be disproportionate. As previously discussed, viral subtype has significant implications for viral virulence and HAART efficacy, and could be an important factor in the present study's outcomes (Spira *et al.*, 2003).

HAART Use

Despite the significant moderator analysis based around treatment group observed in the present research, HAART can still be considered to be a limitation. Including only HAART-naïve patients would have allowed a more sensitive analysis for the present study, and this was indeed the original recruitment protocol. However, due to the relative paucity of these patients at the time of recruitment, and the subsequent impact upon recruitment rate, it was decided that patients taking a HAART regimen should also be included, and the effects of this variable on outcomes were tested. HAART is designed to reduce VL counts to near-undetectable levels, and its efficacy is assessed on its ability to do so (Deeks, 2006). Therefore, the inclusion of HAART-taking patients effectively invalidated the use of VL data as an outcome for the main analysis. Moreover, the inclusion of HAART-taking patients presented further issues for analysis; for example the necessity of measuring HAART adherence. As adherence is vital to HAART efficacy, but is complicated by heavy pill-burden, unpleasant side-effects, drug toxicity and tolerance (Montessori *et al.*, 2004; Wood *et al.*, 2003); whether a patient adhered or not had potentially very large implications on the immunological assessments being recorded.

Not only did the inclusion of HAART-taking patients present more methodological and statistical difficulties, it also compromised the validity of the present findings. Levelling a HAART-naïve patient against a HAART-experienced patient in analysis could be construed as at the very least impractical due to the associated effects that HAART has on HIV immunology (i.e. repopulation of T-cells and increasing VL

suppression) irrespective of the duration of HIV infection (Autran, 1999; Deeks, 2006). The trajectory of immune cell loss versus VL increase would be markedly different between a non-treated and a treated patient, which is a potential explanation for the marginal significance of the main effect. Nevertheless, the effects of both HAART and adherence to it were examined in the present study. The inclusion of HAART-treated patients was nonetheless identified as a moderating factor, which has ultimately added to current knowledge on HL-immunity research, and so although it was a limitation in terms of the original intended protocol, it has provided a novelty to the present research.

There may be additional implications for including a HAART-treated sample, in terms of whether the patient is taking a first- or second-line regimen, what chemical classes of drugs they are taking and whether or not they have had treatment interruptions. It was beyond the scope of the present study to analyse the respective regimens of each patient in detail and to consider this in the analysis, due to the sample size. Thus it is possible that several between-subjects differences on these important issues could have impacted upon the overall findings. For example, immunological outcomes of someone who has just started their first HAART regimen may be markedly different from those of an individual who has been on the same regimen for a few months even during that same period of time. Similarly, an individual who is taking a second-line HAART regimen (i.e. after first-line treatment fails) may not have the same immune reconstitution potential as someone who is about to start their first line of treatment. Further research employing both HAART-treated and HAART-naïve patients is clearly needed, in addition to more exacting control over the line, type and duration of the treatment.

Circumstantial Limitations

The time constraints for the present study resulted in several compromises to the methodological *ideal*. Firstly, the period of study was significantly shorter than that which has been indicated by prior research (e.g. Gruzelier *et al.*, 1996); and indeed that which was originally intended. The precedent for 30 months from the Gruzelier study may perhaps not have been necessary; however a follow-up period of 12 months would have permitted not only more data collection points, but also a greater observable change. As previously outlined, HIV treatment guidelines suggest patients should be monitored every three to four months to adequately monitor HIV

pathogenesis trajectory and treatment efficacy (AIDS Education & Training Centers National Resource Center, 2009). Therefore, the use of three data collection points spanning approximately 6 months was not necessarily inadequate, but would constitute a minimum for assessing disease progression in HIV.

Moreover, due to the restricted actual follow-up time, it was necessary to employ a retrospective artificial baseline to establish a longer total surveillance period. The largest methodological compromise for this restricted data collection period was in the use of a medically retrospective baseline (i.e. using medical data that predated the baseline collection of all other measures). The statistical tests employed for the present data analyses relied heavily upon the factoring-in of baseline immune parameters, which did not coincide with the true baseline for all other parameters, and therefore the findings must be viewed with caution. For example, it is possible that the previously discussed relations between mood and HIV progression were not found because of the discrepancy between the time when the immunological surveillance began and the time when the mood measurement was conducted.

Future Directions

Whilst the present findings suggest some support for the hypothesis of HL predicting prognosis in HIV-1, with the important moderator of HAART-treatment, the discussed observations and study limitations necessitate further research into the relationship between HL and immunity in HIV. The consideration of ethnicity within the context of HIV research is imperative, as Africans disproportionately represent the majority of HIV cases worldwide, as well as having some of the least financial and infrastructural capabilities to cope with the virus burden. Whilst the ethnicity-based moderator analysis ultimately yielded non-significant results, the basis for conducting that analysis was sound, and could potentially provide significant results in a larger sample. Further, and perhaps in accordance with that line of enquiry, analysis of these effects in different viral subtypes could provide an interesting avenue of research; not least to understand why some viral subtypes present more virulence than others. It is possible that these interact with HL in relation to outcomes. Identifying a moderator in HAART treatment has provided new knowledge in to the clinical relevance of HL in HIV disease; however the interpretation of these findings is far from clear-cut. What is not clear is why HAART-treated patients may not be subject to HL influence, and whether different stages in HAART treatment (i.e. first- or second-line, or even

salvage treatment) may present even further differences. Further analysis in to those immune indices that are affected in the same way by both HL and HAART (e.g. lymphocyte proliferation, neutrophil function, or circulating IL-10) together with HIV-specific immune outcomes ($CD4^+$, $CD8^+$, VL) could provide further insight in to why we would not see HL effects on patients treated with HAART. Outside of the HIV context, it may also be interesting to evaluate the influence of HL on CRP and NKCA due to the discrepant effects of left HL and HAART on these immune measures in HIV patients. Does the role of the left hemisphere also incorporate a "decision-taking" action in immunological allostasis?

Of most importance is the need for replication of the present study with a larger sample, for a longer period of follow-up, with a broader profile of ethnicities and a more detailed account of HAART-treatment amongst patients.

The exploratory analyses conducted with the present data also imply further effects of HL in the disease course of HIV, via behaviour. Although the present analyses did not yield any significant effects, it would also be interesting to assess the implication of HL on HAART adherence, which could perhaps be attainable with a larger sample of HAART-treated participants. The observed relationship between HL and the reported number of sexual partners is a new finding, and has important implications for HIV prevention strategies and sexual health initiatives outside of HIV. The reported associative trend with HL and condom use extends the importance of this new finding and its applications in health psychology. The assessment of the effects of a left-HL enhancing cognitive exercise on adherence to condom use, or medication, could prove an interesting and relatively simple interventional strategy, and could be of relevance to $CD4^+$ T-cell counts if the present findings are replicated. Equally, the observed associations between HL and addictive-substance use behaviour could provide an interesting insight in to addiction and rehabilitation sciences.

The reliance on neuropsychological tests for the assessment of HL in the present study provides some circumspection to the results, as they rely on either cognitive or behavioural assessments of this variable. Further, the performance of the line bisection task (LBT) has been argued to rely more on the cerebral organisation of cognitive processes, rather than on unilateral hemispheric activation (Floël *et al.*, 2005). The use of neuroimaging or electroencephalogram (EEG) could provide further clarification of the validity of these measures in this context, as well as providing a potentially more detailed analysis of HL for a replication of the present

study. Indeed, the basis for the present study – the research conducted by the Gruzelier workgroup – employed EEG analyses alongside neuropsychological tests. However, other studies examining the relationship between HL and immunity have also employed neuropsychological approaches with success (e.g. Davidson *et al.*, 1999).

Extraneous to the context of the present study, the field of HL and immunity has been shown to offer a variety of applications in other disease paradigms (Sumner *et al.*, 2011). Applying lateralisation theory to other disease models such as cancer could help to elucidate differential prognosis outside of those factors already identified as being related to disease outcome, given the role of cellular immunity in cancer prognosis (Galon *et al.*, 2006). Furthermore, there is the potential for lateralisation-immunity theory to provide interventions to ameliorate disease outcomes. If, under better conditions, HL can be seen to have a significant impact upon the prognosis of a disease such as HIV, targeted interventions could be devised and implemented in order to attenuate the effects of an unfavourable lateral balance. An interesting example of this comes from a study examining the effects of unilateral rTMS on salivary immunoglobulin A (S-IgA) (Clow *et al.*, 2003). This small study described immunopotentiation (in increased S-IgA concentration) after application of left-sided rTMS, and could have far-reaching effects if it can be replicated in larger samples, and if the role of LHL in HIV observed here, is replicated. Neuropsychological "training" could also be possible to enhance the functioning of one hemisphere, and has already been described in the rehabilitation of stroke patients (Thimm *et al.*, 2009) and the increasing of neural plasticity in older adults (Erickson *et al.*, 2007). Finally, the implication of HL in understanding the efficacy of vaccines may also be another interesting avenue of research. One study has already described increased antibody responses to influenza vaccine in participants given mindfulness meditation, as a function of increased LHL (Davidson *et al.*, 2003). This obviously not only has implications for vaccine development, but also suggests that HL can be enhanced in a clinically meaningful way outside of physical alteration (i.e. rTMS) or neuropsychological training, and may synergistically interact with medical interventions, possibly for the benefit of HIV patients as well.

Chapter 8

Conclusion

THE ROLE OF HEMISPHERIC LATERALISATION IN IMMUNTITY AND HUMAN IMMUNODEFICIENCY VIRUS (HIV) -1

The present thesis sought to evaluate the role of hemispheric lateralisation (HL) in immunity and HIV by the compilation of a systematic review and the execution of an empirical prospective study. To date, a systematic review of human research in to HL and immunity had not been conducted, and so a quasi-meta-analytic review was carried out to evaluate the current available data and the relative strengths of evidence. An empirical research was conducted to examine the relation between HL and the prognosis of an HIV^{+} outpatient sample, modelled on a prior study (Gruzelier *et al.*, 1996) that indicated a relationship despite some key methodological confounds.

Since the first studies into the immunomodulating effects of handedness conducted by Geschwind and Behan (1982), many other research groups have sought to understand the implications of HL on immunity in disease and health. These initial studies of handedness, although conceptually different to HL, provided an insight in to the differential management of the immune system by the brain and inspired research spanning the proceeding three decades. From these initial descriptions of immunity differences between left- and right-handed individuals, animal research conducted by Pierre Neveu and his colleagues expanded these associations from handedness to cerebral asymmetry (Neveu, 1988, 1991, 1992; Neveu *et al.*, 1991; Neveu & Merlot, 2003). Detailing immunological consequences of cortical ablation in animals with paw preference assessment (right- or left-pawed) the extensive studies of Neveu and his workgroups showed asymmetrical, and in some cases opposite, control over interleukin (IL)-1, IL-2, natural killer cell and T-cell proliferation as well as macrophage activation and T-cell mitogenesis (Neveu, 1988, 1991, 1992; Neveu *et al.*, 1991; Neveu & Merlot, 2003). In so doing, he and his colleagues opened up a new field of research combining laterality theory with neuroimmunology. Since then many studies have been conducted with human participants, in both disease and health models using either a handedness or HL paradigm. Some contention has arisen as to the distinction between immunomodulation by handedness effects, and by HL effects; resulting in the separation of these two phenomena in research.

The previously described review of the current literature concerning HL and immunity in humans provided support for differential modulation of the immune system by each hemisphere (Sumner *et al.*, 2011). Amongst the 11 studies evaluated, there was a cumulative consensus that the left hemisphere is immunopotentiating and the right, immunosuppressing. The studies employed a variety of methods to assess the relationship between HL and immunity, in cross-sectional, prospective and experimental examinations. In order to assess the relative strength of the HL-immunity relation in each study, effect size analyses were conducted, which revealed strong statistical HL-immunity associations overall. Summary remarks included the necessity of more rigourous control over third variables in testing the HL-immunity relationship (pertaining to each of the three disciplines contributing to psychoneuroimmunology), the need for replication in larger, more generalisable samples, and indications for more detailed research in locating those areas or processes of the brain that may cause these effects.

One of the reviewed studies examined the effects of HL on immunity prospectively in a disease context (Gruzelier *et al.*, 1996), helping to illustrate an application of laterality-immunity theory in HIV. By examining the effects of HL on immunity in HIV patients, Gruzelier and colleagues described differences in T-lymphocyte levels longitudinally; providing the first insight in to how HL can affect the long-term outcomes of a disease. This finding was important and timely, as it was conducted just before (1996) the advent of the era of highly-active antiretroviral therapy (HAART - 1997) when HIV represented an aggressive, and rapidly fatal illness. During this era, HIV was largely uncontrolled by effective medication, and the epidemic continued to expand across the world, seemingly unabated. Since its discovery HIV has generated a wealth of research to understand its pathogenesis, with a wide variation of disease course being described between individuals (Lama & Planelles, 2007; Langford *et al.*, 2007; Leserman, 2003). A variety of explanations for this variability has been proposed; with psychological, host biological and virological factors all being described as contributing to disease course variability. However, despite three decades of research, these contributing factors have not accounted for all of the variance observed in the disease course of HIV.

The findings from the Gruzelier study, and the combined findings of HL-immunity studies (Sumner *et al.*, 2011), provided another potential factor to explain the

variability in HIV pathogenesis, namely HL. However, the Gruzelier investigation suffered from two key problems in being appropriately applied to HIV disease; several important methodological flaws and its pre-HAART era status. One of the most important methodological short-comings was the lack of control for baseline immunity and other confounders (such as duration of illness, age, gender etc.); a necessity in immunological research. Lack of control for confounders was a key limitation in all the reviewed studies, and indications were made to bring HL-immunity research away from identification, and in to the realms of description – providing control for confounders, and analysing moderating and mediating factors. Further, the Gruzleier study suffered from a small sample size (*N*=27), comprised of only white, homosexual men; with no control for biological or neurological factors which could have impacted upon either HIV or the measurement of HL, or psychological aspects known to impact upon immunity in HIV (i.e. depression, anxiety) (Chida & Vedhara, 2009). Moreover, the study was conducted before the advent of HAART (1997), the most important "game-changer" in HIV since the epidemic began (Simon *et al.*, 2006). This factor is important because although the Gruzelier study only used asymptomatic HIV patients, had the study been conducted more recently some participants may have been within the diagnostic guidelines for HAART treatment. It should be noted that the Gruzelier study sample was drawn from a larger sample of the clinical trials for Zidovudine (one of the first antiretroviral treatments), using both drug- and placebo-taking participants. Although this parallels the present study, insomuch as there is a mixture of drug-taking and drug-naïve patients, HAART is now comprised of many other drug types and classes aside from Zidovudine. Thus it is difficult to use the knowledge gained from their study in today's HIV epidemic, which represents patients from every continent, being treated with some of the most sophisticated batteries of chemical agents developed in recent times (De Clerq, 2009).

Given the strong cumulative evidence of HL's effects on immunity and the precedent for studying HL in HIV patients, a study was devised to extend the findings of the Gruzelier group. Using a larger and more diverse sample of HIV-1^{+} patients (*N*=68), and employing strict controls for potential psychological, biological and neurological influences on the study outcome ($CD4^{+}$ T-cells), the present study sought to extend current knowledge concerning laterality-immunity theory, and variability of HIV

prognosis. The analyses provided some support for the hypothesis of HL predicting HIV prognosis in the whole sample, and an important moderating factor was identified: HAART medication. A significant positive association between left HL and outcome $CD4^+$ T-cell count was observed in the whole sample, independent of confounders (baseline $CD4^+$ T-cell count, HAART medication) employing a basic nonparametric correlation, applying standardised residualisation of $CD4^+$ counts to retain confounder contribution. This finding is in line with the Gruzelier group's study (1996) and with the wider HL-immunity literature (Sumner *et al.*, 2011). However, the initial statistical analysis employing linear multiple regression was unable to provide a significant result for this assessment, which is suggested to be due to skewness within the HL measurement variables, but due to this discrepancy further research is required. Employing HAART medication as a separation factor (moderator) in the participant pool, prospective associations between HL and $CD4^+$ T-cells were observed in patients not taking a HAART regimen, independent of the confounder of baseline $CD4^+$ T-cell count. This important observation was the first of its kind, and its absence in the HAART-treated sub-sample is suggested to be resultant from HAART masking HL effects, as HAART is an immunomodulator in its own right.

Another potential moderator was identified, namely ethnicity, with suggestions that left-HL is predictive of outcome $CD4^+$ count in African, rather than European, patients. It is not possible at this stage to support the notion that ethnicity may be a true moderator in the HL-immune relationship in HIV, as the initially significant results yielded from the multiple regression analyses were not echoed by the subsequent nonparametric correlational analyses. The suggestion of ethnicity being a moderator in the HL-immune relationship is also a new implication, and although it will require more testing before it can be supported as a moderating factor, it has important implications in a global epidemic that is overwhelmingly over-represented in Africa.

Proposed explanations for the conflicting significance of the main analyses (multiple regression versus Spearman's *rho* correlation) include the sample size (which was ultimately slightly under that which was projected as required for such analysis *a priori*), the presence of outliers amongst the HL variable data, and significant skewness and kurtosis amongst these same variables even after outlier exclusion. Additionally, the measures of HL in the present study (self-report and

neuropsychological tests) not only differed from the preceding study, but also from those employed by former studies within the broader discipline that required HL assessment (i.e. not through stroke or surgery) as these all used Electroencephalogram (EEG). It is possible that the two measures employed for the present study may not be as sensitive to *activational* HL as measured by EEG examination, but may be more representative of *functional* HL only. For example, the Line Bisection Task (LBT) relies on cognitive cerebral organisation for perceptual and motor skills rather than on overall hemispheric activation, creating potential operational and interpretation biases (Flöel *et al.*, 2005). Equally, the Hemispheric Preference Test (HPT) relies on the self-report of behaviours and preferences, which have been suggested to be modulated by affective states (Merckelbach *et al.*, 1990; Russo *et al.*, 2001).

Despite the short-comings of the present study, it still fits within the framework of the existing literature concerning laterality and immunity; with left HL being observed to predict a slower decline in T-cells amongst HIV patients. Crucially, the present findings help to understand some proportion of variance between patients in terms of differences in time to morbidity and mortality in HIV disease. Further, the study adds to the current knowledge in the discipline of laterality-immunity research by describing a moderating factor of HAART medication; which has clinical significance in potentially providing a way to identify those patients that may require HAART initiation earlier (i.e. people low on left-HL) as well as identifying groups that would benefit from other targeted interventions. Neuropsychological interventions to increase left hemisphere activity could be implemented with sufficient ease as to be available to people in all areas of the world without relying on costly equipment, further medical involvement, or access to sophisticated clinics or treatment centres. Additionally, the hint toward ethnicity being a moderator in the HL-HIV-immunity relationship is another new suggestion in HL and HIV research. Whilst this suggestion is not a substantiated finding *per se*, it merits further research due to its near-significance and the disproportionate weight of HIV infections, with the equally disproportionate lack of resources to cope with HIV seen, in Africa. Thus, future research needs to test whether cognitive tasks which activate the left hemisphere improve $CD4^+$ T-cell levels in non-medicated HIV patients. Additionally, the exploratory analyses concerning HL and behaviour uncover important implications for sexual health behaviour, HIV clinic attendance and substance misuse. These associations and trends are again new findings within the discipline, and warrant

further exploration, with potential significance to deepen our understanding of these behaviours, their antecedents, and potentially their prevention.

The complitation of a systematic review to analyse the cumulative evidence of HL-immunity research allows the discipline to assess the current knowledge, and advance that knowledge in areas of methodological limitation. The strength of evidence of the reviewed studies suggests that the time for mere observation of this relationship has ended. There is now the need to describe the HL-immune relationship by way of analysing moderating and mediating factors and controlling for known confounders, as well as looking in to the neurobiological mechanisms that facilitate and drive the lateralised brain-to-immune communication. Moreover, the combined suggestions of these data permit a more credible perspective of this often contentious subject area. Whilst it was not possible to conduct a full meta-analysis of the available data, the inclusion of effect size analysis afforded a higher level of scientific application than a simple review, and can be implemented relatively easily if the appropriate data are reported.

In summation, the present thesis contributes a synthesised description of laterality-immunity research in humans, in the systematic review and the advancement of existing research into the effect of HL on HIV progression. The systematic review provides the first cumulative perspective on the current evidence in the laterality-immunity domain of psychoneuroimmunology in humans. Moreover by employing effect size analysis it provides meaningful, *empirical* weight to the sometimes contested role of HL within the field. The advancement of the one study within the literature to examine the practical implications of HL in immunity (i.e. Gruzelier *et al.*, 1996) not only provides further evidence for the immunomodulating effects of HL, but also expands the knowledge to suggest the moderating factor of medication, namely HAART treatment, and the potential moderator of ethnicity. Importantly, the present work may have implications for other immune-mediated diseases, to eventually help guide interventions for diseases without a current cure, and to understand some of the variation observed in disease course, wellness and behaviour.

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APPENDIX A

ETHICAL APPROVAL NOTICES

Ethical approval from Brunel University School of Health Science & Social Care Research Ethics Committee

Approval for amendments to protocol from Brunel University School of Health Science & Social Care Research Ethics Committee

Ethical approval from Universitair Ziekenhuis Brussel/Vrije Universiteit Brussel Commissie Medische Ethiek

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Research Ethics Committee

28 August 2008

Proposer: Rachel Sumner

Title: **Can neuropsychological tests reflecting cerebral lateralisation and cingulate activity predict HIV prognosis?**

Reference: **08/08/PHD/05**

Letter of Approval

The School Research Ethics Committee has considered the amendments recently submitted by you in response to the Committee's earlier review of the above application

The Chair, acting under delegated authority, is satisfied that the amendments accord with the decision of the Committee and has agreed that there is no objection on ethical grounds to the proposed study. Approval is given on the understanding that the conditions of approval set out below are followed:

- *The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee.*

NB:

- **Research participant information sheets and (where relevant) flyers, posters and consent forms, should include a clear statement that research ethics approval has been obtained from the School of Health Sciences and Social Care Research Ethics Committee.**
- **Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.**

Elizabeth Cassidy
Chair Research Ethics Committee
School of Health Sciences and Social Care

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Research Ethics Committee

9 July 2010

Approval of Amendment to Protocol

Proposer: Rachel Sumner

Title: Can neuropsychological tests reflecting cerebral lateralisation and cingulate activity predict HIV prognosis?

Reference: 08/08/PHD/05

The School Research Ethics Committee has considered the amendment to protocol recently submitted by you in relation to a new sampling collaboration with University Hospital Brussles, and amendments to the inclusion criteria and procedures, as detailed in your written communication received on 8th July 2010. Acting under delegated authority, the Chair is satisfied that there is no objection on ethical grounds to the amendments. In addition, the Committee has approved a 'Raising Awareness' PowerPoint presentation that will be used at the Terrence Higgins Trust. Approval is given on the understanding that the conditions of approval set out below are followed:

- *A permission letter, as proof of collaboration, from the University Hospital Brussles, confirming the details of the collaboration, is received by the School Research Ethics Committee before the amendment to protocol is implemented.*
- *Proof of local Research Ethics approval is received by the Committee before implementation*
- *Any changes to currently approved documentation with regard to Information Sheets and/or Consent Forms, other than translation, must be received by the Committee before implementation of the amendments.*
- *The agreed protocol must be followed. Any changes to the protocol will require prior approval from the Committee.*

NB:

- **Research participant information sheets and (where relevant) flyers, posters and consent forms, should include a clear statement that research ethics approval has been obtained from the School of Health Sciences and Social Care Research Ethics Committee.**
- **Approval to proceed with the study is granted subject to receipt by the Committee of satisfactory responses to any conditions that may appear above, in addition to any subsequent changes to the protocol.**

David Anderson-Ford
School Research Ethics Officer
School of Health Sciences and Social Care

Universitair Ziekenhuis Brussel

Vrije Universiteit Brussel

COMMISSIE MEDISCHE ETHIEK (O.G. 016)

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DR. P. LACOR
AIDS-REFERENTIECENTRUM
UZ BRUSSEL

Brussel, 18-11-2010

Ons Kenmerk : **2010/188**

ADVIES VAN DE COMMISSIE MEDISCHE ETHIEK

Betreft :

Does Hemispheric Lateralization Predict Prognosis in HIV Patients not on HAART?
B.U.N. 14320109236

Na kennis genomen te hebben van de documenten betreffende het bovenvermeld project, inclusief de aangepaste versies van het informatie- en toestemmingsformulier in het Nederlands, Frans en Engels, ontvangen op 17-11-2010 en aangepast aan de opmerkingen in het advies van 23 september 2010, besluit de Commissie Medische Ethiek tijdens haar zitting van **18 november 2010**

DAT DE VOORZIENE STUDIE MAG ONDERNOMEN WORDEN.

Deze goedkeuring blijft geldig voor de duur van het project. De Commissie wenst een jaarlijks overzicht van de stand van zake van het project te ontvangen. De studieresultaten dienen overgemaakt te worden aan de Commissie bij het beëindigen van de studie. Zij herinneren de verantwoordelijke van het experiment eraan dat dit experiment onder zijn persoonlijke verantwoordelijkheid zal worden uitgevoerd. Het gunstig advies van de Commissie betekent geenszins dat de Commissie de verantwoordelijkheid van het experiment op zich neemt. De Commissie Medische Ethiek werkt en is georganiseerd volgens de richtlijnen van GCP.

Met de meeste hoogachting,

P. Devroey, voorzitter

Cc: FAGG, Departement R&D, t.a.v. Mevr. Musch Eurostation blok 2, Victor Hortaplein 40 /40 - 1060 Brussel

Universitair Ziekenhuis Brussel

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DR. P. LACOR
AIDS-REFERENTIECENTRUM
UZ BRUSSEL

Brussel, 21-01-2011

Ons Kenmerk : **2010/188**

ADVIES VAN DE COMMISSIE MEDISCHE ETHIEK

Betreft :

Does Hemispheric Lateralization Predict Prognosis in HIV Patients not on HAART?
B.U.N. 14320109236

Nieuwe protocoltitel:

Does Hemispheric Lateralization Predict Prognosis in HIV Patients?

De Commissie Medische Ethiek nam kennis van, en verleent een gunstig advies aan, het amendement betreffende de aanpassing van de inclusie- en exclusiecriteria in het protocol van bovenvermelde studie (uitbreiding naar deelname van patiënten on HAART) en betreffende de wijziging van de titel van de studie.

Ter uwer informatie :
Tijdens haar vergadering van 26/03/'98 besliste onze Ethische Commissie het volgende : *"De voorzitter (en in zijn afwezigheid de ondervoorzitter) mag, zonder de Commissie te raadplegen, beslissingen nemen i.v.m. aanvullende gegevens of ongewenste effecten die voor sommige dossiers later dan de oorspronkelijke aanvraag zouden ingestuurd worden . Mocht een punt van majeur belang blijken te zijn dan moet hij dit op de dagorde van de eerstvolgende commissievergadering plaatsen."*
Dit betekent dat de documenten enkel aan de voorzitter werden voorgelegd.

Met de meeste hoogachting,

P. Devroey, voorzitter

Cc: FAGG, Departement R&D, t.a.v. Mevr. Musch Eurostation blok 2, Victor Hortaplein 40 / 40, 1060 BRUSSEL

APPENDIX B

INSTRUMENTS & MATERIALS

English language version of participant information sheet

English language version of research consent form

Hospital Anxiety & Depression Scale
(Zigmond & Snaith, 1983)

Hemispheric Preference Test (Zenhausern, 1978)

Mini Mental State Examination (Folstein *et al.*, 1975)

Morisky Medication Adherence Scale (Morisky *et al*., 1986)

Can Differences in Brain Function Predict HIV Prognosis?

The study you are being asked to take part is conducted by Rachel Sumner, a PhD student at Brunel University in West London. The study is being used as the main research to complete this course. The Brunel School of Health Sciences and Social Care ethics committee has approved this research. The study is being supervised by Dr Alex Nowicky of Brunel University and is being run in conjunction with Professor Yori Gidron and the cooperation of University Hospital of Brussels.

The contact details of the main researcher, her supervisor and the chair of the research ethics committee are supplied below; please make a note of them for future reference.

- *What is it about?*

The current study concerns whether it is possible to predict how people progress in their course of HIV by looking at differences in brain functioning. In order to assess this we will be asking you to complete some questionnaires and some simple tests to look at how your brain works. In order to assess how this impacts upon your prognosis we will need to collect your $CD4^+$ and viral load at three and six months after you have completed the initial part of the study. Please note that we will only count on reports of these measures and will not be asking you to undergo any additional blood tests. The easiest way for us to do this is to access your medical records when you have your normal check ups.
In order to carry out the study we must have access to your medical record. We will only collect information concerning your $CD4^+$ and viral load, and that information will only be collected by a member of the research team. All of your medical information will be kept strictly confidential, however if you do not feel comfortable allowing us access to your medical records then please do not participate.

- *What will I need to do?*

The first part of the study will involve completing questionnaires to assess your suitability to take part in the study. As we are looking for some very specific relationships between your brain functioning and your illness there are certain conditions whereby you may not be suitable to participate, this is nothing to be alarmed about it is purely a reflection of the very early stages of this line of research.

The main study involves answering a questionnaire about your background and the various aspects of your illness, such as how and when you contracted HIV, what symptoms you have and what types of treatment you access. The questions on this questionnaire are personal, but will remain confidential and are only included because they are essential to our being able to understand the relationship between any other

personal factors (eg. health behaviour, duration of illness etc.) and the course of your illness.
The next part of the study involves completing questionnaires and computer-based tests which look at your mood, your health behaviours and how your brain functions. There are two types of neuropsychological test for you to complete; one presents you with a bisected line and asks you to indicate which half of the line is greater: the left or the right. The other test asks questions concerning your thoughts and activities and you are asked to select one of the two statements that most applies to you.

These questionnaires and tests should take between 20 and 30 minutes to complete. If you are not sure you have time to complete them all in one sitting then please book an appointment with the researcher. Should you wish, it may be possible for you to complete this study at home, using our secure web server for the tests and having a brief telephone call. However, as this study originates from the UK you should be confident in your ability to speak, read and understand English if you choose this option.

The questionnaires are intended for us to collect information only, and as such we cannot use them to provide you with any form of diagnostic information. If there is anything in the tests that causes you concern you are urged to speak to your doctor or clinician. You are also asked to stop your participation if you feel any type of distress during the proceedings of this research.

- *What next?*

As mentioned previously, we will need to obtain your $CD4^+$ count and viral load at three and six months after your initial visit with the researcher. With your permission this will be done directly with the staff at the hospital. Please note that all members of the research team, including those who are assisting us at the hospital, are bound by confidentiality and no other details aside from your CD4 and viral load will be disclosed.
Because we are trying to examine a relationship between your brain function and the course of your illness, we may also want to collect information about your CD4 and viral load up to six months prior to you beginning the study. Again, this is the only information we will collect from your medical records and it will remain strictly confidential.

- *Ethical conduct & your rights in research*

You have the right at any time during or after this research to withdraw yourself and your data from the study. This right is comprehensive and enduring; should you wish to discontinue your participation you can do so at any time and without providing a reason. If you choose to withdraw yourself from the study you can request your data or you can request that it be destroyed.
All the information you provide us is confidential and anonymous. You will be given a "participant identification code" when you agree to take part, which will ensure your anonymity and allows us to identify your data should you wish to withdraw. All of the data you provide us will include this unique code, and only one file will include a link between this code and your name. This file will be kept in a locked box in the researcher's home.

The information gleaned from this research will be prepared in to a doctoral thesis, scientific journals and conference presentations. At no point will any individual personal information be made available to the public.

We are conducting this study with the generous cooperation of the University Hospital Brussels. Whether or not you decide to participate in this research will have no bearing whatsoever on your access to their facilities or the quality or degree of care that you receive from them. Any member of staff at The University Hospital Brussels who is not a member of the research team will not be notified of any individuals' participation in the research, but they are aware of the content and background of the study should you wish to discuss any aspects of the study with them. If you feel any degree of concern about the impact this study may have on your illness or your use of clinical services we urge you to discuss this with the experimenter or your clinician.

If you have any doubts about any part of the research, or you do not feel completely comfortable in carrying out what we ask you to do then please do not take part. We understand that we are asking you to take part in research about something that is deeply personal to you, and we respect your right to privacy.

This study has been approved by the Brunel University School of Health Sciences and Social Care Research Ethics Committee.

- *Contact Information*

Rachel Sumner, MSc. - Researcher
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Dr Alex Nowicky Ph. D. - Supervisor
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Informed Consent

Research Consent
(Please tick as appropriate)

1. I have received sufficient information about this research to understand what my participation will involve.

   YES □
   NO □

2. I give consent for the research team to access my medical records to obtain information about my CD4 count and viral load

   YES □
   NO □

3. I understand that any information I provide will be anonymous and confidential.

   YES □
   NO □

4. I understand that I have the right to withdraw myself and my data from this research at any time and without necessarily providing reason for doing so.

   YES □
   NO □

By completing the below information as indication of the above declarations I confirm that I am willing to participate in this research based on the information I have received. I understand that my personal information will be completely confidential and that any data collected from me will be anonymous. I also understand that I have the right to withdraw myself and my data from this research at any point during the research or after it has finished.

Please provide us with your name and date of birth. This will not be attached to any of the information you provide us, but will need to be retained should you at any point wish to withdraw yourself and your data from the research.

Name……

Date of Birth ……. (DD/MM/YYYY)

This study has been approved by the Brunel University School of Health Sciences and Social Care Research Ethics Committee.

Demographic Questionnaire

Age……

Gender : Male Female

Ethnicity:

Education:

Employment status:

Sexual orientation:

Relationship status:

(a) How many sexual partners have you had in the last 12 months?

(b) Condom use: 12 months?

Always Often Sometimes Never

(c) STI? – last 12 m?

	Frequency of use in the last month				
	No use	Once or twice	Once a week	Several times a week	Daily
Alcohol					
Cigarettes/Tobacco					
Marijuana					
Cocaine					

HIV for …….years and ……months

MoC:

Moriskey:

Meds:

Hospital Anxiety & Depression Scale

Please read the statements and tick which response best suits your experience.

	Yes definitely	Yes sometimes	No, not much	No, not at all
1. I wake up early and then sleep badly for rest of the night.				
2. I get very frightened or have panic feelings for apparently no reason at all.				
3. I feel miserable and sad.				
4. I feel anxious when I go out of the house on my own.				
5. I have lost interest in things.				
6. I get palpitations, or sensations of “butterflies” in my stomach or chest.				
7. I have a good appetite.				
8. I feel scared or frightened.				
9. I feel life is not worth living.				
10. I still enjoy the things I used to.				
11. I am restless and can’t keep still.				
12. I am more irritable than usual.				
13. I feel as if I have slowed down.				
14. Worrying thoughts constantly go through my mind.				

Hemispheric Preference Test

1. How often are your decisions based on objective facts rather than feelings?
Never (1) → Always (10)

2. Are you psychic?
Never (1) → Always (10)

3. Do you like using symbols and/or images in solving problems?
Never (1) → Always (10)

4. How good are you at teaching and/or explaining by manipulating objects?
Never (1) → Excellent (10)

5. Are you artistically or musically creative?
Never (1) → Always (10)

6. Are you logical?
Never (1) → Always (10)

7. How good are you at solving crossword puzzles?
Poor (1) → Excellent (10)

8. How quickly can you read?
Very slowly (1) → Very Quickly (10)

9. How vivid are your daydreams?
Not at all vivid (1) → Extremely vivid (10)

10. How good is your ability to think of synonyms for words?
Poor (1) → Excellent (10)

11. Do you remember your dreams often?
Never (1) → Always (10)

12. How vivid are your dreams?
Not at all vivid (1) → Extremely vivid (10)

13. Are you fluent in using words?
Never (1) → Always (10)

14. Do you use a playful approach to solving problems?
Never (1) → Always (10)

15. Do you use a serious, all-business approach to solving problems?
Never (1) → Always (10)

16. Do you like experiences to be planned and structured?
Never (1) → Always (10)

17. Do you like to think or read while sitting upright?

Never (1) → Always (10)
18. How often does your thinking consist of words?
Never (1) → Always (10)

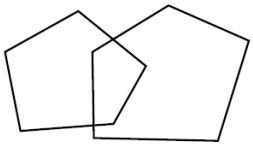
19. How often does your thinking consist of mental pictures or images?
Never (1) → Always (10)

20. Do you like teaching or explaining by visual presentation?
Never (1) → Always (10)

MINI MENTAL STATE EXAMINATION (MMSE)

Patient's name:

Hospital number:

ONE POINT FOR EACH ANSWER	DATE			
ORIENTATION				
Year Month Day Date Time	____/5	____/5	____/5	____/5
Country Town District Hospital Ward	____/5	____/5	____/5	____/5
REGISTRATION				
Examiner names 3 objects (eg apple, table, penny) Patient asked to repeat (1 point for each correct). THEN patient to learn the 3 names repeating until correct.	____/3	____/3	____/3	____/3
ATTENTION AND CALCULATION				
Subtract 7 from 100, then repeat from result. Continue 5 times: 100 93 86 79 65 Alternative: spell "WORLD" backwards - dlrow.	____/5	____/5	____/5	____/5
RECALL				
Ask for names of 3 objects learned earlier.	____/3	____/3	____/3	____/3
LANGUAGE				
Name a pencil and watch.	____/2	____/2	____/2	____/2
Repeat "No ifs, ands, or buts".	____/1	____/1	____/1	____/1
Give a 3 stage command. Score 1 for each stage. Eg. "Place index finger of right hand on your nose and then on your left ear".	____/3	____/3	____/3	____/3
Ask patient to read and obey a written command on a piece of paper stating "Close your eyes".	____/1	____/1	____/1	____/1
Ask the patient to write a sentence. Score if it is sensible and has a subject and a verb.	____/1	____/1	____/1	____/1
COPYING				
Ask the patient to copy a pair of intersecting pentagons: 	____/1	____/1	____/1	____/1
TOTAL	____/30	____/30	____/30	____/30

Moriskey Medication Adherence Scale (Moriskey *et al.*, 1986)

	YES	**NO**
a) Do you ever forget to take your medicine?		
b) Are you careless at times about taking your medicine?		
c) When you feel better do you sometimes stop taking your medicine?		
d) Sometimes if you feel worse when you take the medicine, do you stop taking it?		

APPENDIX C

RAW DATA

Skewness and Kurtosis reports for HL and $CD4^+$ collections

Multiple Regression analyses – for the whole and moderator-split samples

Exploratory behavioural analyses

1. Skewness and Kurtosis in HL variables and $CD4^+$ collections

Variable	Skewness (Std. Error)		Kurtosis (Std. Error)	
Left HPT (Log_{10} transformation)	-2.455	(.306)	14.55	(.604)
Left LBT	-.257	(.291)	-.730	(.574)
$CD4^+$ T1	.823	(.306)	.452	(.604)
$CD4^+$ T2	.572	(.295)	.137	(.582)
$CD4^+$ T3	.766	(.309)	1.094	(.608)

2) Multiple Regression analysis – whole sample, using confounders identified *a priori* for the outcome measure of $CD4^+$

Warnings

For models with dependent variable CD4 +1, the following variables are constants or have missing correlations: HAART. They will be deleted from the analysis.

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Mean Clinic Visit, Non_Adh_2, CD4 - 1, Duration of HIV[a]	.	Enter
2	LHPT_Log_2[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: CD4 +1

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.873[a]	.763	.732	138.39085	.763	24.898	4	31	.000
2	.875[b]	.766	.727	139.66292	.003	.438	1	30	.513

a. Predictors: (Constant), Mean Clinic Visit, Non_Adh_2, CD4 -1, Duration of HIV

b. Predictors: (Constant), Mean Clinic Visit, Non_Adh_2, CD4 -1, Duration of HIV, LHPT_Log_2

ANOVA[c]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1907392.731	4	476848.183	24.898	.000[a]
	Residual	593712.825	31	19152.027		
	Total	2501105.556	35			
2	Regression	1915933.654	5	383186.731	19.645	.000[b]
	Residual	585171.902	30	19505.730		
	Total	2501105.556	35			

a. Predictors: (Constant), Mean Clinic Visit, Non_Adh_2, CD4 -1, Duration of HIV

b. Predictors: (Constant), Mean Clinic Visit, Non_Adh_2, CD4 -1, Duration of HIV, LHPT_Log_2

c. Dependent Variable: CD4 +1

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-407.397	175.662		-2.319	.027
	CD4 -1	.889	.098	.831	9.088	.000
	Duration of HIV	.552	4.708	.011	.117	.907
	Non_Adh_2	53.775	37.041	.131	1.452	.157
	Mean Clinic Visit	2.485	.847	.257	2.934	.006
2	(Constant)	-1045.837	980.977		-1.066	.295
	CD4 -1	.911	.104	.851	8.748	.000
	Duration of HIV	-.107	4.855	-.002	-.022	.982
	Non_Adh_2	57.023	37.702	.138	1.512	.141
	Mean Clinic Visit	2.485	.855	.257	2.907	.007
	LHPT_Log_2	202.409	305.886	.062	.662	.513

a. Dependent Variable: CD4 +1

Excluded Variables[b]

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics
						Tolerance
1	LHPT_Log_2	.062[a]	.662	.513	.120	.878

a. Predictors in the Model: (Constant), Mean Clinic Visit, Non_Adh_2, CD4 -1, Duration of HIV

b. Dependent Variable: CD4 +1

3) Multiple Regression analysis – whole sample, using confounders identified statistically for the outcome measure of $CD4^{+}$.

Variables Entered/Removed

Model	Variables Entered	Variables Removed	Method
1	HAART, CD4 -1[a]	.	Enter
2	LB_LHL, LHPT_Log_2[a]	.	Enter

a. All requested variables entered.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.846[a]	.716	.704	138.07832	.716	60.569	2	48	.000
2	.852[b]	.726	.702	138.70150	.009	.785	2	46	.462

a. Predictors: (Constant), HAART, CD4 -1

b. Predictors: (Constant), HAART, CD4 -1, LB_LHL, LHPT_Log_2

ANOVA[c]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2309575.283	2	1154787.642	60.569	.000[a]
	Residual	915149.893	48	19065.623		
	Total	3224725.176	50			
2	Regression	2339772.333	4	584943.083	30.405	.000[b]
	Residual	884952.843	46	19238.105		
	Total	3224725.176	50			

a. Predictors: (Constant), HAART, CD4 -1

b. Predictors: (Constant), HAART, CD4 -1, LB_LHL, LHPT_Log_2

c. Dependent Variable: CD4 +1

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	172.218	82.534		2.087	.042
	CD4 -1	.850	.081	.820	10.514	.000
	HAART	-72.759	49.378	-.115	-1.474	.147
2	(Constant)	-293.632	841.962		-.349	.729
	CD4 -1	.860	.083	.830	10.396	.000
	HAART	-60.863	50.890	-.096	-1.196	.238
	LHPT_Log_2	160.093	266.439	.048	.601	.551
	LB_LHL	-7.956	7.782	-.080	-1.022	.312

a. Dependent Variable: CD4 +1

Excluded Variables[b]

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics
						Tolerance
1	LHPT_Log_2	.058[a]	.724	.473	.105	.933
	LB_LHL	-.085[a]	-1.107	.274	-.159	.989

a. Predictors in the Model: (Constant), HAART, CD4 -1

b. Dependent Variable: CD4 +1

4) Bivariate correlations for exploratory behavioural analyses using the Line Bisection Test (LBT)

Correlations

		Non_Adh_2	12m No. Sexual Partners	Condom Use 12m	Alcohol use 1m	Cigarette/Tobacco use 1m	Marijuana use 1m	Cocaine use 1m	Mean Clinic Visit
LB_LHL	Pearson Correlation	.020	-.220	.132	.174	-.097	-.256*	.130	-.035
	Sig. (2-tailed)	.895	.071	.287	.157	.430	.035	.290	.784
	N	47	68	67	68	68	68	68	65

*. Correlation is significant at the 0.05 level (2-tailed).

5) Bivariate correlations for exploratory behavioural analyses using the LBT, with the sample split by ethnicity

Correlations

Ethnicity			Non_Adh_2	12m No. Sexual Partners	Condom Use 12m	Alcohol use 1m	Cigarette/Tobacco use 1m	Marijuana use 1m	Cocaine use 1m	Mean Clinic Visit
European	LB_LHL	Pearson Correlation	.127	-.356*	.108	.024	-.211	-.357*	.120	.115
		Sig. (2-tailed)	.495	.017	.487	.876	.163	.016	.432	.468
		N	31	45	44	45	45	45	45	42

African	LB_LHL	Pearson Correlation	-.161	.078	.148	.334	.[a]	.[a]	.[a]	-.360
		Sig. (2-tailed)	.551	.725	.502	.119	.	.	.	.092
		N	16	23	23	23	23	23	23	23

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

6) Bivariate correlations for exploratory behavioural analyses using the Hemispheric Preference Test (HPT) for the whole sample.

Correlations

		Non_Adh_2	12m No. Sexual Partners	Condom Use 12m	Alcohol use 1m	Cigarette/Tobacco use 1m	Marijuana use 1m	Cocaine use 1m	Mean Clinic Visit
LHPT_Log_2	Pearson Correlation	-.020	.032	-.046	-.104	.030	-.015	.177	-.034
	Sig. (2-tailed)	.899	.808	.730	.430	.823	.910	.176	.800
	N	44	60	59	60	60	60	60	57

7) Bivariate correlations for exploratory behavioural analyses using the HPT, with the sample split by ethnicity

Correlations

Ethnicity			Non_Adh_2	12m No. Sexual Partners	Condom Use 12m	Alcohol use 1m	Cigarette/Tobacco use 1m	Marijuana use 1m	Cocaine use 1m	Mean Clinic Visit
European	LHPT_Log_2	Pearson Correlation	-.017	.094	-.137	-.151	.130	.023	.253	-.052
		Sig. (2-tailed)	.931	.566	.406	.351	.425	.889	.115	.760
		N	30	40	39	40	40	40	40	37
African	LHPT_Log_2	Pearson Correlation	-.004	.262	.349	.315	.[a]	.[a]	.[a]	-.017
		Sig. (2-tailed)	.990	.264	.131	.176	.	.	.	.942
		N	14	20	20	20	20	20	20	20

a. Cannot be computed because at least one of the variables is constant.