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The response of the central auditory system to sound in normal hearing adults with and without HIV/AIDS: An fMRI study

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(Dr Oliver Sacks)

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"Trust in the Lord with all your heart, and lean not on your own understanding, in all your ways acknowledge Him, and He shall direct your paths."

(Proverbs 3:5-6)

- John A Logan

ABSTRACT

Titel: The response of the central auditory system to sound in normal hearing adults with and without HIV/AIDS: An fMRI study

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Research applying fMRI to evaluate any activation in the central auditory nervous system (CANS) is currently limited, but has shown some promising potential. fMRI is likely to become the tool of choice for addressing many research questions concerning the auditory system, especially regarding the localization of the function of certain auditory areas and brain connectivity.

The main aim was to determine the response of the central auditory nervous system (CANS) to sound in normal hearing adults with and without HIV/AIDS, using fMRI.

A between-group comparative design was used to determine and compare the response of the CANS to auditory stimuli of a sample of 15 normal hearing participants without (HIV/AIDS) and 12 normal hearing participants with (HIV/AIDS).

Structural and fMRI images were acquired using a 1.5T Siemens Magnetom Espree. Two different listening stimuli tasks namely nonsense syllables and warble tones were presented binaurally using earphones. These stimuli were presented with a block design with two conditions, silence (baseline) of twenty seconds and the stimuli task tone of twenty seconds. It was interleaved with duration of 20 seconds per block, with each block repeated four times. The task paradigm was done with blood oxygen level-dependent (BOLD) technique.

Images were analysed by statistical parametric mapping software. A priori regions of interest (ROIs) for the CANS were defined and separate participant analyses were performed on the average signal in each ROI using the general linear model. The beta values generated from this analysis reflected the mean percentage signal change within the ROI of the CANS for the two tasks for each participant. A whole brain analysis was also done to determine and compare the multiple areas of activation and responses to the two tasks in the whole brain.

Both groups of participants showed activation in all the ROIs of the CANS, but the mean percentage signal change in BA41 and BA42 differed significantly between the 2 groups with a p-value of 0.03 both BA41 and BA42. The control group showed a significantly greater increase in neuronal activity caused by cerebral blood flow to BA41 and BA42 than the HIV/AIDS group.

The influence of possible confounding factors such as gender, age, and education on the mean percentage signal change in BA41 and BA42 in the two groups was also determined. Age ($p = 0.95$) and education ($p = 0.68$) had no statistically significant effect on the mean percentage signal change in BA41 and BA42 for any of the two groups in this study. However, gender had a significant effect for the mean percentage signal change for BA41 ($p = 0.042$) and BA42 ($p = 0.037$).

The influence of the CD4 count and years on ART on the neural response of the ROIs CANS to the auditory stimulation (nonsense syllables) was also determined. The results showed a negative linear regression analysis, thus indicating an inverse correlation between the years on ART and the activated BOLD signals in BA41 and BA42 areas during the nonsense syllables task. The use of ART could have reduced the CD4 count in this study. Although this trend was observed, it would require a bigger sample to shed more light on these findings.

Results of the response of the ROIs to warble tones indicated no significant differences between all the ROIs of the two groups, however a borderline significant difference (0.07) of activation occurred, namely the inferior colliculus in the right hemisphere of the HIV/AIDS group.

The results of the whole brain analysis showed that the two participant groups differ in brain activation and confirmatory voxel-by-voxel comparison results were noted. These noted differences could be related to the neurocognitive impairments related to HIV/AIDS, but were not explored in depth and should therefore be investigated and documented further.

This study indicates that BOLD fMRI provides the possibility to display brain regions responding to specific auditory stimuli applied during the scanning session. It thus has become an essential tool for studying human auditory function. As these differences could not have been observed with any other basic audiometric test procedure in people with and without HIV/AIDS.

These findings support the idea that HIV/AIDS can affect the CANS and support the potential of auditory fMRI to assess the CANS in HIV/AIDS people. Therefore, auditory fMRI will play an important role in the development of new techniques for clinical application in the near future.

Keywords:

auditory functional Magnetic Resonance Imaging (fMRI), Blood Oxygenation Level Dependent (BOLD), Central Auditory Nervous System (CANS), Cluster of Differentiation 4 (CD4), Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome (HIV/AIDS)

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CHAPTER 1
INTRODUCTION AND ORIENTATION

"If the human brain were so simple that we could understand it,
we would be so simple that we couldn't."
(Pugh, 1977:154)

1.1. Introduction

This chapter provides an introduction to human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) its effect on the human body, and specifically on the auditory system. The purpose of this chapter is to present an orientation to the study and its background, and explain the problem statement and rationale for the study. In addition, Chapter 1 presents a clarification of terminology and abbreviations, provides the design and structural outline of the six chapters of the thesis, and ends with a conclusion.

1.2. Orientation to the study and background

There is an increasing incidence of HIV/AIDS infection worldwide. It was estimated in 2015 that approximately 7 000 000 [6 700 000 - 7 400 000] people in South Africa were living with HIV/AIDS. This constitutes the world's largest population infected with HIV/AIDS (UNAIDS, 2016). The HIV/AIDS form a worldwide pandemic with devastating consequences that affect the lives of many people of all ages (Swanepoel & Louw, 2010). The HIV is a retrovirus that enters the host (the human body) through different body fluids. It is transmitted by sexual intercourse, blood transfusion, the use of intravenous drugs, or perinatal transmission. It serves as the aetiologic agent of AIDS and causes the onset of opportunistic infections that can affect the brain. It can also affect the hearing mechanism, causing various types of hearing impairments such as hearing loss (Quidicomo & Matas, 2013).

HIV enters the host CD4 cell and triggers the onset of AIDS – primarily in the immune cells containing the CD4 cells. When the HIV cells multiply, HIV invades the body and destroys these cells, causing the human body's defenses to deteriorate. The infected cells are T-lymphocytes T4, which develop in the bone marrow and ensure the production of antibodies that supplement the body's immune system. The CD4 cells are important for the immune system's ability to fight infectious diseases. The CD4 count (which is important for monitoring a person's immune status) is measured in CD4+ cells. The classification of the clinical stage of HIV depends on the CD4+ cell count (Evian, 2003).

A lower CD4 count indicates that the body has a weakened immune system, and as a result will become susceptible to opportunistic infections. These infections and diseases, commonly related to HIV (hereinafter 'HIV/AIDS'), are caused by bacteria, viruses, protozoa, fungi, and malignancies (Evian, 2003). According to Cohen and Berger (2007) and Moayedi (2010), these conditions can have specific head and neck manifestations, including the following:

- **Central nervous system:** Toxoplasmosis, Cryptococcus, progressive multifocal leukoencephalopathy
- **Oropharanx:** Oral candidiasis, oral ulcerations, oral hairy leukoplakia
- **Larynx:** Laryngeal candidiasis, histoplasmosis, lymphomas, Kaposi's sarcoma
- **Nose and sinus:** Sinusitis, allergic rhinitis, nasopharyngeal lymphoid hypertrophy
- **Ear:** Infections can affect the external auditory canal and cause otitis media, conductive and mixed hearing loss, opportunistic infections, and ototoxicity that leads to sensorineural hearing loss and vestibular disorders causing vertigo, dizziness, or disequilibrium.

One of the possible manifestations of HIV/AIDS is a change in hearing (Campanini, Marani, Mastroianni, Cancellieri & Vicini, 2005). The literature indicates that HIV/AIDS can infect the auditory pathway from the external ear to the cortical level (Matas, Angrisani, Magliaro & Segurado, 2014). Pathologies caused in the auditory pathway may include otitis externa, otitis media, vertigo, and vestibular disorders. HIV/AIDS could also directly infect the brain and cause neurological diseases linked to central processing deficit (Maro, Moshi,

Clavier, MacKenzie, Kline-Schoder, Wilbur, Chambers, Fellows, Jastrzembski, Mascari & Buckey, 2014).

It is clear that the hearing deficits associated with HIV/AIDS can be either sensorineural or conductive, and can occur at different locations along the auditory pathway. Various causative factors have been identified and discussed in the literature.

a) Infections such as otitis media, mastoiditis, meningitis (Matas et al., 2014)

Infections caused by HIV/AIDS have the potential to affect a person's hearing in different ways. Otitis media is commonly found in HIV/AIDS patients (Chandrasekhar, Connelly, Brahmbhatt, Shah, Kloser & Baredes, 2000). The most commonly found changes to the central nervous system are AIDS encephalopathy, subacute encephalitis, cryptococcal meningitis, central nervous system toxoplasmosis, tuberculosis meningitis, and bacterial and viral meningitis that can often lead to sensorineural hearing loss (Matas, Silva, Marcon & Gonçalves, 2010). A hearing disorder could be the result of any of these illnesses that can damage the central auditory nervous system (CANS) and the hearing mechanism (Matas et al., 2010). Otorhinolaryngological manifestations are common in individuals with HIV/AIDS, it is herefore important for early diagnosis and treatment to provide improvement for these people with HIV/AIDS.

b) The use of anti-retroviral drugs or anti-tuberculosis drugs (ototoxic medication) (Matas et al., 2014)

Antiretroviral treatment (ART) medication is currently the only treatment that controls the disease's progression. However, ARTs can cause undesirable ototoxic side-effects that can negatively impact the hearing and consequently the quality of life of the person who depends on the treatment (Assuiti, Lanzoni, Dos Santos, Erdmann & Meirelles, 2013). Medications used to treat disseminated TB can likewise carry the risk of contributing to hearing loss, since such medication is also known to be ototoxic (Gurney & Murr, 2003). Persons with HIV/AIDS, especially those who do not receive treatment, experience a high rate of HIV/AIDS-associated encephalopathy. Even in persons receiving treatment there is still substantial concern about continued central nervous system involvement, given its compartmental nature and the differential pharmacokinetic distribution of ARVs.

Neurocognitive deficits are still found in persons with HIV/AIDS, despite active ART (Mirza & Rathore, 2012). The benefits of treatment should therefore be evaluated or assessed against the side effects, since hearing loss can have a significant negative impact on an individual's life (Torre III, Cook, Elliott, Dawood & Laughton, 2016).

c) The direct effect of HIV/AIDS or other viruses on neural pathways and structures (Matas *et al.*, 2014)

HIV/AIDS can also affect the brain parenchyma, i.e. the functional tissue in the brain, leading to AIDS dementia, as well as the pathways involved in the central processing of auditory information (York, Franks, Henry & Hamilton, 2001). Research indicates that about 75 per cent of adults with HIV/AIDS can experience different types of hearing problems in different stages of the HIV/AIDS disease as a direct result of these influences of HIV/AIDS on the hearing mechanism (Matas et al., 2014). Auditory abnormalities associated with HIV/AIDS and its treatment has been reported in persons with varying degrees of HIV/AIDS infection, both symptomatic and asymptomatic patients, and in patients on ART (Khoza-Shangase, 2010; Matas et al., 2014; Campanini et al., 2005; Quidicomo et al., 2013). Furthermore, patients with HIV/AIDS can experience a 20 to 40 per cent hearing loss caused by pathologies of the outer ear, middle ear, or inner ear, or by opportunistic infections or treatment using ototoxic medications (Matas et al., 2014; Van der Westhuizen, Swanepoel, Heinze & Hofmeyr, 2013). Due to the neurological diseases consequential to HIV/AIDS, moreover, a person might develop a central hearing disorder or even an auditory neuropathy (Stearn & Swanepoel, 2010)

Although HIV/AIDS is both directly and indirectly responsible for various changes to the ear and hearing, there is no clear relationship between disease progression and severity of auditory manifestations. The effects of HIV/AIDS on the auditory system differ greatly from one person to the next. To date, no large-scale study has, in fact, indicated which of these potential causes predominate, or how frequently hearing problems have been experienced by persons with HIV/AIDS (Maro et al., 2014). As knowledge about the influence of HIV/AIDS on the hearing mechanism and the concomitant functional implications is still limited, there is a need for more in-depth and encompassing auditory examinations to shed more light on this aspect (De Lange, 2008).

The requisite auditory examinations would ideally involve collecting data from large numbers of people living with HIV/AIDS, and would involve well-trained audiologists capable of both gathering and interpreting such data. Hearing data of persons infected with HIV/AIDS can be collected using various instruments or equipment. The following procedures and techniques can be employed and have been used in research and in the clinical setting:

a) Pure tone air-and-bone audiometry

An audiological evaluation usually commences using pure tone audiometry to determine the degree and type of hearing loss. Both HIV/AIDS and its treatment can cause difficulty in recognising and interpreting sounds, however, even though audiometry results could be normal (Maro et al., 2014).

b) Speech audiometry

Speech audiometry forms an important part of the audiometric test battery as a crosscheck for other tests and also to determine the site of lesion in the auditory system. The purpose of a Speech Discrimination test is to determine the speech understanding of the participant and to rule out retrocochlear damage (Debonis & Donohue, 2004). Speech audiometry can give information about CANS and establish whether any pathology is involved (Stach, 2010).

c) Immittance testing (tympanometry)

Tympanometry measures the integrity of the middle ear, and how efficiently it can transform acoustic energy into mechanical energy. Research studies involving manifestations of HIV/AIDS and hearing loss used acoustic immittance measures (tympanometry) to determine the status of the tympanic membrane and to identify any abnormality or pathology of the middle ear (Matas et al., (2014); Harris, Bardien, Schaaf, Petersen, De Jong & Fagan, (2012); Buriti, Oliveira & Muniz, (2013)). This is especially important as it was found that people infected with HIV/AIDS present with a higher incidence of the middle ear pathology (Kohan, Rothstein & Cohen, (1988); Chao, Czechowicz, Messner, Alarcón, Kolevic Roca, Larragán Rodriguez, Gutiérrez Villafuerte, Montano & Zunt, (2012); Assuiti et al., (2013)).

d) Otoacoustic emission (OAE)

OAE testing can be used to evaluate cochlear functioning. With OAE, the influence of ototoxic medication on the outer hair cells of the cochlea can be detected before it is evident on the pure audiogram. Reports on research about cochlear function in adults are limited. In the study by Torre III et al., (2016), OAE testing was used to evaluate associations between HIV/AIDS status, HIV/AIDS treatment, and the cochlear status. OAEs measure only cochlear function and not hearing sensitivity.

e) Auditory brainstem response (ABR)

Persons with HIV/AIDS tend to have a higher percentage of altered auditory brainstem responses (ABRs). This suggests that there is a higher incidence of impairments in the CANS, in persons who are HIV/AIDS positive that are not found in persons who are HIV/AIDS negative. These alterations imply impairment in the lower brainstem as well as early detection of neuropathology associated with HIV/AIDS (Matas, Samelli, Angrisani, Magliaro & Segurado, 2015). In the study of Mata et al., (2015) there were no significant differences between HIV/AIDS people using ART treatment or not.

In the study by Serafini, Stagni, Chiarella, Brizi & Simoncelli (1998) ABR was used to evaluate the nerve transmission integrity in the course of HIV/AIDS infection. Significant impairments in nerve transmission were shown from the earliest stages of HIV/AIDS infection and worsened as the disease progressed. These results suggest that the upper part of the brainstem may be the main target of involvement.

Using ABR in people with HIV/AIDS can be useful for the detecting early signs of neurodegeneration. These findings also highlight the role of immunosuppression in the development of neural abnormalities involving the brainstem and the auditory pathway during the progression of the disease (Matas, Samelli, Magliaro & Segurado, 2017). The study of Matas et al., (2017) suggested that auditory information was impaired in the cortical and subcortical regions, supporting the need for more detailed investigation of the auditory function in people with HIV/AIDS. Alteration in this population includes subcortical demyelination, even before clinical neurological manifestations are present. Researchers utilize the use of ABR and P300. P300 provides an early indicator of cognitive deficits in HIV/AIDS, reduced

amplitude suggests lower attention, and longer latency a slower processing of information. Longer latencies regarding waves I, III and V as well as interpeak I-V for ABR, suggesting lower speeds of neuroelectric impulse transmission throughout the brainstem auditory pathway. Longer P300 latency, suggesting reduced processing speed of auditory information in cortical regions. A reduction in the amplitude of the Pa wave in MLR in the comparison of RGII with RGI, suggesting alterations to the auditory pathway in cortical and subcortical regions (Matas et al., 2017)

Altough all of the above tests are valuable in identify pathology at the peripheral auditory system **t**he use of imaging techniques such as fMRI might shed more light on the pathology occurring in the central auditory areas.

f) Auditory fMRI

Functional MRI (fMRI) can be a useful tool to detect pathology in the CANS earlier than when using ABR. fMRI produces a different outcome, and alterations can be found in this population before the onset of clinical symptoms such as neurological and cognitive deficits (Matas et al., 2015). In Maro et al., (2014), the data suggests that more complete assessments of the central auditory function are needed for persons with HIV/AIDS, as it is currently not possible to determine definitively whether the central auditory findings are related primarily to the HIV/AIDS infection or to ART.

The study of Maro et al., (2014) shows that either HIV/AIDS infection or its treatment could be a possible cause of the difficulty in auditory processing (recognising sounds) despite the relatively normal values shown by audiometry testing. Clinical audiologists typically use audiometric testing for the diagnosis of hearing impairment. None of the current guidelines recommend imaging testing. Resourceful clinicians have, however, taken note of the fact that techniques have been developed which enable researchers to visualise the brain and how it functions. fMRI may be used more often in research and in clinical practice to classify the auditory processing pathways and to identify abnormalities in the pathway (Micallef, 2015).

The acoustic signal is transformed by the middle ear, the inner ear, the nuclei in the brain stem, and the thalamus; the information then arrives in the cortex. At every level of the process, the neural pattern relating to a specific sound is communicated by the neurons situated

in that region of the brain. Functional imaging techniques afford researchers an indirect view of the auditory activity of the brain in humans. These techniques are non-invasive, and it is possible to determine auditory-related activity throughout the whole brain over a given period of time (Hackett & Kaas, 2004).

fMRI can be used to identify auditory processing disorders (APD) in areas where there is either an abnormality or no neural activity. The use of fMRI, when combined with the patient's history and audiometric results, has been proved to give a more evidence-based diagnosis than history and audiometric results alone (Micallef, 2015). However, fMRI of the auditory nervous system presents unique challenges. Several factors can influence the results: the intensity, frequency, rate, and duration of the task, and the habitation and cognitive confounds of the stimulus. These must be controlled to avoid potential difficulties when obtaining and interpreting the results of an examination. When doing the examination, the challenge is to ensure that the task is accurate, not complex, precise, reproducible, and easy to perform with the participant remaining still during the entire process. For this reason, in most of the studies in auditory fMRI, tones, syllables, and words were used so that the stimuli were basic. Reproducible music can also be used as a stimulus of the auditory area (Suzuki, Kitano, Kitanishi, Itou, Shiino, Nishida, Yazawa, Ogawa & Kitajima, 2002).

Modern brain imaging techniques have had a great impact on the study of the human central auditory function. Magnetic resonance imaging (MRI) has become one of the most significant non-invasive methods for investigating human brain structure and functional changes. It has a much-improved spatial sensitivity and specificity, and it can map auditory responses with sufficient topographic detail. With the use of fMRI, multiple observations can be done on the same patient, and the results can justify the investigation of longer-term dynamic processes, such as functional changes after disease, damage, retraining, or therapy (Hall, Lanting & Hartley, 2014). Although functional imaging of the central auditory pathway has not been widely used in ear, nose and throat or audiology departments, a growing literature on its potential application is being developed.

Insights gained from functional imaging research with normal-hearing subjects can help to develop efficient protocols to study patients with hearing problems (Hall et al., 2014). To date, not enough auditory fMRI research has been performed to determine the influence of HIV/AIDS on the central auditory system. fMRI can highlight those areas that are abnormal

or have no neural activity, and should contribute positively to the field of auditory examination and the understanding of the consequences of HIV/AIDS (Micallef, 2015).

Zhan, Buckey, Fellows, & Shi, (2017) reported a systematic review of the literature to evaluate the evidence for HIV/AIDS affecting parts of the central nervous system involved in central auditory processing. These findings support the idea that HIV/AIDS can affect the central auditory pathways and support the use of central auditory tests to assess the central nervous system.

People with central auditory deficits may have neurological defects in the pathways from the auditory nerve through to the higher auditory pathways in the brain. These deficits can cause difficulty with understanding speech in background noise despite normal auditory thresholds, and have problems with localising sound or recognising voices. The central auditory system includes the cochlear nucleus, trapezoid body, lateral lemniscus and superior olivary complex in the brainstem, the inferior colliculi in the midbrain, the medial geniculate nucleus in the thalamus, the internal capsule in the basal ganglia region and the auditory cortex. Damage to these structures can cause central auditory processing.

1.3. Problem statement and rationale

The preceding discussion clearly points out the current predicament: while it is acknowledged that HIV/AIDS can affect the CANS, the resultant changes have not yet been sufficiently investigated and documented. Research aimed at determining the response of the CANS to sound in persons with HIV/AIDS could ultimately contribute to better diagnosis and better service delivery.

During the past 20 years, neuroscience of the auditory system has progressed so far that we are now able to understand the functional structure of the auditory system in normal hearing persons as well as in persons with hearing loss. Imaging methods are suitable for use with both children and adults. In the case of fMRI, several observations can be conducted with the same person, and accuracy is improved by mapping the activation of the brain structure specific to that patient (Hall, 2007). fMRI measures the brain function and neural activity for longer than a specific time range in a picture format (Huettel, Song & McCarthy, 2009).

Brain function and brain changes have become significant areas of research in the field of audiology. Lanting, De Kleine, Eppinga & van Dijk, (2010) used auditory fMRI to investigate functional changes in the auditory system in order to determine non-auditory areas that might relate to the generation and perception of tinnitus. A study by Langers, van Dijk, Schoenmaker & Backes, (2007) aimed to gain insight into the functional changes that can occur in the CANS as a result of peripheral hearing loss.

An electronic database was utilized to identify and obtain research articles from peer-reviewed literature relevant to the current study. These articles provide background information related to auditory fMRI and HIV/AIDS. Table 1 reflects the database and the search strategy employed. The Pubmed database search used four approaches to find relevant literature: (1) auditory fMRI-related articles, (2) auditory fMRI articles about people infected with HIV/AIDS, (3) auditory fMRI-related articles published during the past five years, and (4) auditory fMRI articles published during the past five years of people infected with HIV/AIDS.

Table 1: Database and search strategy details

Database	Search strategy	Identifiers	Results
Pubmed	Auditory fMRI. The terms occurred in the title, keywords and/or abstract of articles.	"auditory fMRI"	7424
Pubmed	Auditory fMRI and HIV/AIDS persons. The terms occurred in the title, keywords and/or abstract of articles.	"auditory fMRI and HIV/AIDS"	16
Pubmed (2011 to 2016)	Auditory fMRI. The terms occurred in the title, keywords and/or abstract of articles.	"auditory fMRI"	2365
Pubmed (2011 to 2016)	Auditory fMRI and HIV/AIDS persons. The terms occurred in the title, keywords and/or abstract of articles.	"auditory fMRI and HIV/AIDS"	2

Many reports cover **auditory fMRI**, but very few relate to **auditory fMRI of persons infected with HIV/AIDS**. During the search only 16 reports about **auditory fMRI and HIV/AIDS** could be found, and only two of these reports were published during the period 2011 to 2016**.** None of these reports, however, investigated the same issue as this study. Rather, they focused on HIV/AIDS in the brain and magnetic resonance imaging (MRI).

Thus far, auditory fMRI examinations have not been used extensively for routine clinical purposes in persons infected with HIV/AIDS. The literature search revealed that only two research reports or articles on **auditory fMRI and persons living with HIV/AIDS** have

been published – nationally or internationally – to determine the influence of HIV/AIDS on the central auditory system. The literature also indicated that tests were done on lesion sites at the peripheral and brainstem levels, but that investigations at the central level are still lacking (Paken, 2007).

The use of fMRI for routine diagnostic evaluation is not recommended because of its high cost and relatively limited availability. However, non-invasive fMRI in research settings can provide valuable information on the pathophysiology of HIV/AIDS infection within certain brain areas such as the auditory system, and on the potential neurotoxic actions of the ART. Obtaining this fMRI information about the auditory system will help to diagnose hearing problems due to central auditory involvement appropriately, and also help to ensure that these patients receive suitable rehabilitation for their hearing disorders (Ances, Vaida, Yeh, Liang, Buxton, Letendre, McCutchan & Ellis, 2010).

In the last few years, investigations into auditory perception and language have been enhanced by using fMRI that specialises in localisation of the central responses. fMRI has advanced the understanding of the response and organisation of the regions in the brain that are activated by the perception of sounds. fMRI is the tool of choice for many neuroscientists to understand the neural response. Against this background the neuroimaging techniques such as fMRI in people with HIV/AIDS can give an understanding into the mechanisms underlying the neuro part of HIV/AIDS. This could monitor the HIV/AIDS disease in progression and may assist in evaluating the efficacy of particular ART treatments (Masters & Ances, 2014).

The aim of the current study was to determine the response of the Central Auditory Nervous System (CANS) to sound in normal hearing adults with and without HIV/AIDS, by using functional magnetic resonance imaging (fMRI). The following research questions were posed:

- What is the fMRI response of the auditory nervous system to sound in normal hearing adults with and without HIV/AIDS?
- What are the effects of HIV/AIDS, and of antiretroviral medication, on the fMRI response in the auditory nervous system?

1.4. Clarification of terminology and abbreviations

Since the same words or terms may have different connotations in various disciplines, and certain terms may not be equally familiar to all professionals, a clarification of the terms used in this research report is in order.

- **Acquired Immunodeficiency Syndrome (AIDS)**

A disease where there is a severe loss of the body's cellular immunity, greatly lowering the resistance to infection and malignancy. AIDS develops as a result of HIV/AIDS infection, producing constant immune dysfunction and deterioration (Bekker, 2010).

- **Antiretroviral therapy (ART)**

Antiretroviral therapy is used (with strict adherence) in the medical treatment of HIV/AIDS/AIDS (Bekker, 2010). The ART used in South Africa includes generic fixed-dose combinations, such as Tenofovir, Emtricit-abine, and Efavirenz (Bekker, Venter, Cohen, Goemare, Van Cutsem, Boulle & Wood, 2014).

- **Blood oxygenation level dependent (BOLD)**

Blood oxygenation level dependent (BOLD) contrast imaging is a method used in fMRI: it measures brain activity by detecting changes associated with blood flow. The technique relies on the fact that cerebral blood flow and neuronal activation are coupled: when an area of the brain is in use, blood flow to that region also increases (Huettel et al., 2009).

- **Central auditory nervous system (CANS)**

The term refers to the auditory neural pathway from the cochleae to the auditory cortex. When sound waves have been converted to neural signals in the last section of the external auditory system, they are transmitted through cranial nerve VIII to reach different anatomical structures where the neural information is further processed. These anatomical structures include the cochlear nucleus (pons), the superior olivary complex (pons), lateral leminiscus

(pons), inferior colliculus (midbrain), the medial geniculate body (thalamus) and the auditory areas of the cerebrum (Bellis, 2008). Fibre tracts connect the two sides of the ascending pathway at several levels, in such a manner that each ear projects more to the contralateral temporal cortex. As sensory information travels within the CANS, processing occurs not only in a serial order but also in a parallel manner. The result is a highly efficient and redundant system (Demanez & Demanez, 2003).

- **Central auditory processing (CAP)**

The central auditory processes are the auditory system mechanisms and processes. CAP describes what happens when the brain recognises and interprets sounds (sound localisation and lateralisation), auditory discrimination, auditory pattern-recognition, and temporal aspects of audition (Bellis, 2008).

- **Central auditory processing disorders (CAPD)**

CAPD is an umbrella term for a variety of disorders that affect the way the brain processes auditory information. People may have normal hearing ability, but the information is encoded and processed differently by the brain (Katz, Stecker & Henderson, (1992); Moore, 2015). It also describes various difficulties in the ability to discriminate between, identify, or comprehend auditory stimuli, particularly when the auditory signal is in any way degraded, in spite of normal peripheral hearing (Phillips, 2002).

- **Cluster of Differentiation 4 (CD4)**

CD4 is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages and dendritic cells. This measurement indicates the health of the immune system. CD4 molecules bind on the surface of the T-lymphocytes. CD4 cells are destroyed by HIV/AIDS, but they are not replaced quickly enough, and as a result the immune system CD4 count decreases (Bekker, 2010).

- **Functional magnetic resonance imaging (fMRI)**

Functional magnetic resonance imaging (fMRI) is a functional neuroimaging procedure using MRI technology that measures and maps brain activity by detecting and measuring the changes in blood oxygenation over time (Huettel et al., 2009).

- **Human Immunodeficiency Virus /Acquired Immune Deficiency Syndrome (HIV/AIDS)**

This is the infection that is caused by a retrovirus that becomes incorporated into the human lymphocytes and macrophages, resulting in the erosion of the human immune system (Bekker, 2010).

- **Magnetic Resonance Imaging (MRI)**

Magnetic resonance imaging (MRI) is a technique that uses a magnetic field and radio waves to create detailed images of the organs and tissues within the body. Most MRI machines are large, tube-shaped magnets. When a person is positioned inside an MRI machine, the magnetic field temporarily realigns hydrogen atoms in the body and uses magnetic fields to produce the images (Huettel et al., 2009).

- **Opportunistic Infections (OI)**

These are infections caused by pathogens (bacteria, viruses, fungi, and protozoa) that take advantage of a host with a weakened immune system. HIV/AIDS in its advanced stages is often associated with the manifestation of opportunistic infections (Mohammed & Nasidi, 2006).

1.5. Outline of the thesis

The primary focus of this study is to determine the response of the central auditory nervous system (CANS) to sound in normal hearing adults with and without HIV/AIDS, using functional magnetic resonance imaging (fMRI).

Chapter One: Introduction and orientation

This chapter serves as an introduction to the research field and research project by providing background on the HIV/AIDS pandemic and a brief but systematic overview of the significant manifestations of HIV/AIDS in the human auditory system. It also provides a brief overview of the development of auditory fMRI – a non-invasive method – and gives a rationale for the study. The problem statement, leading to the research question, defines the rationale for this research. The layout of the chapters and the definitions of terms used are also discussed.

Chapter Two: Literature study

In Chapter Two the auditory system is explained, along with the influence of HIV/AIDS on the human immune system and the hearing mechanism. The theoretical underpinnings and principles of magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI) are provided, based on the existing theoretical knowledge and literature.

Chapter Three: Methodology, data collection, and analysis

In Chapter Three, the design, material, apparatus, and procedures used to select the subjects are discussed. The material and apparatus for data collecting, recording, and analysis are reviewed. Ethical considerations are also considered, as are the measures implemented to ensure the reliability and validity of the study.

Chapter Four: Results

Chapter Four includes the quantitative results obtained from each statistical analysis conducted during data collection. The results are presented to correspond with the sub-aims set out in Chapter Three.

Chapter Five: Discussion of results

In this chapter the findings are discussed and interpreted in depth, followed by a thorough description of the value and meaning of these results in relation to the existing literature.

Chapter Six: Conclusion, clinical implications and recommendations

In this final chapter, the conclusions derived from the results are summarised. The study is evaluated in terms of its validity, reliability, and clinical value, and recommendations made for future research. The limitations of the study are discussed, and all the main findings of the study are summarised.

1.6. Conclusion

A brief and general overview of the study has been provided in this chapter. This has enabled the researcher to lay the foundations for the text that follows, and provided an orientation to the study, the problem statement, the problem question, the aims and objectives of the study, and the scope of the thesis. Furthermore, the structure of the thesis has been set out with a brief exposition of the six chapters.

This study was undertaken to determine the extent of CANS involvement in participants with and without HIV/AIDS by using fMRI. Establishing the value of using fMRI in this way can add to the knowledge base of HIV/AIDS and its effect on the auditory system. There are different ways to investigate the auditory system. fMRI is the most effective method, but unfortunately is the least used. This is primarily because fMRI is not widely used in South Africa.

Thus the results of this study could not only benefit the patients themselves, but also be of value to the professionals who serve the patients, and broaden the knowledge base of this field of study locally and internationally.

The next chapter will present a literature review of the influence of HIV/AIDS on the auditory system, and of the principles of MRI and fMRI.

CHAPTER 2
THE INFLUENCE OF HIV ON THE AUDITORY SYSTEM AND THE PRINCIPLES OF USING AUDITORY FMRI

"The extent of its global reach and its pervasive and devastating nature has ensured that HIV/AIDS is the health care challenge of our time. Its effects are far reaching and impact not only those affected, but also the wider family, community, and societal structures."
(Swanepoel & Louw, 2010:1)

2.1. Introduction

HIV/AIDS is a pandemic not only in South Africa but also worldwide. It affects millions of people of all ages (Swanepoel & Louw, 2010). Despite the gains made in the fight against the HIV/AIDS pandemic over more than three decades it is still the biggest challenge for the healthcare system in this country (Posel, Kahn & Walker, 2007).

Chapter two briefly reviews the structure and functioning of the auditory system, including the central auditory pathway. The chapter also presents a theoretical overview of the influence of HIV/AIDS on the auditory system and the typical audiological manifestations. Finally, the chapter includes the principles of magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI), as well as the value of the procedure in the auditory assessment of CANS.

2.2. HIV/AIDS and the human body

HIV/AIDS has a detrimental effect on the whole human body and accordingly can cause hearing related problems, such as hearing loss, tinnitus, vertigo and changes in the peripheral auditory pathway. It is therefore necessary to investigate these hearing and hearing related changes so as to determine how they relate to HIV/AIDS (Assuiti et al., 2013).

When such abnormalities manifest, they must be treated as soon as possible so as to improve the quality of life of the person involved (Matas, Leite, Magliaro & Concalves (2006); Quidicomo et al., 2013)

2.2.1. Epidemiology of HIV/AIDS

HIV/AIDS is a global pandemic and in 2015 about 36.7 million people lived with HIV worldwide (see Figure 1). In 2015 in Eastern and Southern Africa about 19.1 million people lived with HIV (UNAIDS, 2016). The life expectancy of people living with HIV is increasing and this can be attributed to an improved accessibility to public health education, awareness programmes, health care facilities and also the availability of ART medication (Heinze, 2014).

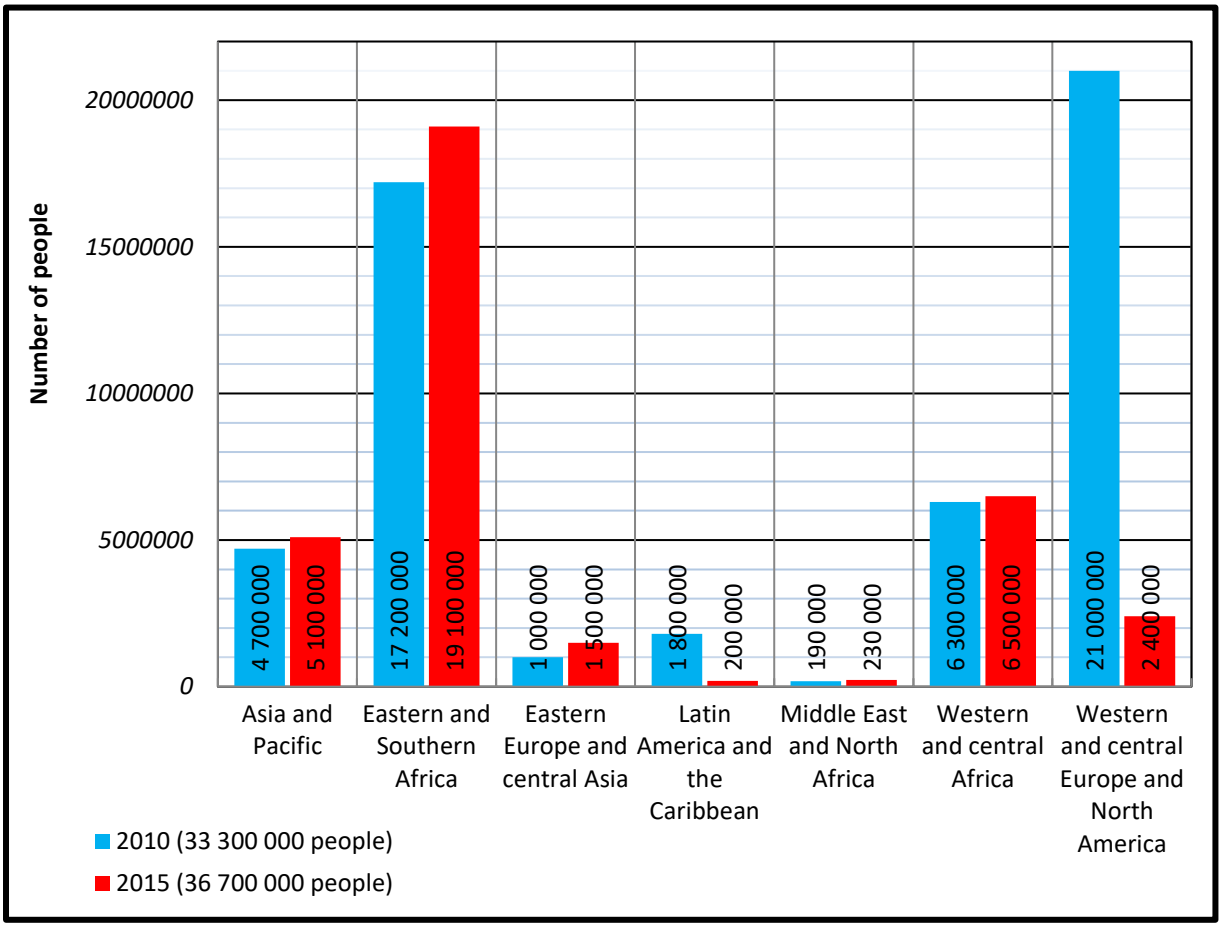


Figure 1: Global HIV/AIDS epidemic estimates by region

The graph in Figure 1 demonstrates that, in 2015, about 19.1 million people in the eastern and southern Africa region lived with HIV/AIDS. The eastern and southern Africa region consist of 21 countries: Angola, Botswana, Comoros, Eritrea, Ethiopia, Kenya, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Rwanda, Seychelles, South Africa, South Sudan, Swaziland, Uganda, United Republic of Tanzania and Zimbabwe. It is the region that is worst affected by the HIV/AIDS epidemic in the world (UNAIDS, 2016).

During 2015 about 7 million of the 19.1 million people living with HIV/AIDS were to be found in South Africa. This is the biggest and most high profile of the HIV/AIDS epidemic in the world (AVERT, 2015). Women represent approximately half (51%) of the total worldwide population of adults living with HIV/AIDS. Gender inequalities, differential access to service, and sexual violence increase women's vulnerability to HIV/AIDS. In sub-Saharan Africa, young women account for 63% of young people living with HIV/AIDS (UNAIDS, 2016).

2.2.2. The response of the immune system to HIV/AIDS

HIV/AIDS is transmitted by sexual intercourse, blood transfusion, the use of intravenous injection of drugs, occupational injury to the body, and perinatal transmission between mother and child (Quidicomo et al., 2013; Matas et al., 2014). HIV/AIDS attacks the human body's immune system, which should produce white blood cells and antibodies that counter viruses and bacteria. It is an etiological agent that causes substantial immunological impairment in the whole human body. The cells that fight the infection are termed CD4 cells (also known as T-cell lymphocytes) and the CD4 cell count indicates the status of the immune system. HIV/AIDS binds with and penetrates into the CD4 cells in the form of a host, then attacks these cells and makes "copies" of itself (duplicates itself) in an effort to destroy the cells. A high concentration of the HIV/AIDS-virus is then distributed into the human body. The CD4 cells can be replaced but only over a period of time. During this process the immune system will be exhausted and the CD4 cell numbers will decrease (Bekker, 2010).

As the HIV/AIDS spreads / circulates through the human body, it changes the functioning of the immune system. This brings about weakening of the body defences and leads to a situation where the vulnerable body is susceptible to the onset of a series of diseases caused by organisms and opportunistic infections (Quidicomo et al., 2013; Matas et al., 2014).

Table 2 provides a summary of common opportunistic infections, diseases, and causing agents associated with HIV/AIDS.

Table 2: Summary of common opportunistic infections

	Causing agent/cancer	Opportunistic infection
Bacteria	Mycobacterium tuberculosis	Lungs, meninges
	Group B streptococcus	Lungs
	Haemophilus influenzae	Lungs
	Pneumococci	Lungs and bloodstream
	Salmonella	Gastrointestinal tract and blood
	Atypical mycobacterium	Lung and other organs
Viruses	Herpes simplex	Skin and nervous system
	Herpes zoster (shingels)	Skin and nervous system
	Cytomegalovirus	Lungs, retina, brain, gastrointestinal tract, liver
Protozoa	Toxoplasmosis	Meninges, brain, eyes
	Cryptosporidium	Gastrointestinal tract and gall bladder
Fungi	Candida	Mouth, eosophagus, gastrointestinal tract, vagina, skin, nails
	Cryptococcus	
	Histoplasmosis	Meninges and lungs
	Pneumocystis jiroveci	Lungs
Cancers	Kaposi's sarcoma	Skin, gastrointestinal tract
	Lymphomas- non-Hodgkin's	Lymph nodes
	Ano-genital (human papilloma virus)	Ano-genital area
	Liver (associated with hepatitis B or C	Liver
	Multi-centre (Castleman's disease)	

Adapted from Evian (2003)

The information in Table 2 reflects that the common HIV/AIDS related opportunistic infections and diseases are caused by bacteria, viruses, protozoa, fungi and malignancies (cancers). People with HIV/AIDS are more susceptible to contracting these diseases, because their immune systems are most vulnerable to infections. HIV/AIDS is a progressive disease and occurs in stages of severity. An overview of the various classification and staging systems of the HIV/AIDS disease progression will be described in the next section.

2.2.3. HIV/AIDS classification: CDC and WHO staging systems

HIV/AIDS staging and classification systems are used to track and monitor the HIV/AIDS epidemic. These systems provide clinicians and individuals living with HIV/AIDS with essential information about the progression and clinical management of HIV/AIDS.

The two main classification and staging systems currently in use worldwide are the Centers for Disease Control and Prevention (CDC) classification system and the World Health Organization (WHO) Clinical Staging and Disease Classification system (Buehler & Berkman, 1993; CDC, 1993; WHO, 2016).

The WHO classification system

Clinical staging is useful not only at the time of diagnosis of the disease, but also in the follow-up of treatment regimens once the individual has been diagnosed with HIV/AIDS. It also serves as a guideline for when to start administering ARTs or other HIV/AIDS-related treatments and is particularly useful in settings where testing to determine CD4+ cell counts is not available. Table 3 lists the four WHO stages of HIV/AIDS in adults with confirmed HIV/AIDS infection. Specific clinical symptoms, diseases, and conditions are defined in each stage.

Table 3: WHO clinical staging associated with clinical symptoms of HIV/AIDS

Clinical stage	HIV/AIDS related symptoms, diseases and conditions	
Clinical Stage 1	Asymptomatic	
	Persistent generalized lymphadenopathy (PGL)	
Clinical Stage 2	Moderate unexplained weight loss	Recurrent respiratory tract infections (RTIs, sinusitis, bronchitis, otitis media, pharyngitis)
	Herpes zoster	
	Angular cheilitis	
	Recurrent oral ulcerations	Popular pruritic eruptions
	Seborrheic dermatitis	Fungal nail infections of fingers
Clinical Stage 3	Unexplained severe weight loss (>10% of presumed or measured body weight)	Unexplained persistent fever for >1 month (>37.6°C, intermittent or constant)
	Unexplained chronic diarrhea for >1 month	Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia)
	Persistent oral candidiasis (thrush)	
	Pulmonary tuberculosis (current)	Oral hairy leukoplakia
	Neutropenia (neutrophils <500 cell/μL)	Unexplained anemia (hemoglobin <8 g/dL)
	Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis	Chronic thrombocytopenia (platelets <50 000 cells/μL)
Clinical Stage 4	**Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations**	
	HIV wasting syndrome	Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration)
	Oesophageal candidiasis	
	Pneumocystis pneumonia	
	Extrapulmonary TB	Kaposi's sarcoma
	Recurrent severe or radiological bacterial pneumonia	Central nervous system (CNS) toxoplasmosis HIV encephalopathy
	Conditions where confirmatory diagnostic testing is necessary	
	Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes). Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)	Extrapulmonarycryptococcosis including meningitis Disseminated non-tuberculous mycobacteria infection Progressive multifocal leukoencephalopathy (PML) Candida of trachea, bronchi or lungs Cryptosporidiosis
	Isosporiasis	Visceral herpes simplex infection
	Recurrent non-typhoidal salmonella septicaemia	Lymphoma (cerebral or B cell non-Hodgkin)
	Invasive cervical carcinoma	Visceral leishmaniasis

Adapted from WHO (2016)

The CDC classification system

The Centers for Disease Control and Prevention (CDC, 1993) use a revised system to classify HIV/AIDS disease and infection. This system is based on the CD4+ cell counts and presence of HIV/AIDS-related conditions and symptoms to categorize the severity of the disease and determine appropriate clinical management. According to the US CDC definition, an individual has AIDS if he/she is infected with HIV/AIDS and presents with a CD4+ T-cell count below 200 cells/ mm^3 (or a CD4+ T-cell percentage of total lymphocytes of less than 14%), **or** he/she has one of the defining illnesses listed in the CDC description.

It is known that the CD4+ cell count determines the health of the immune system. Reduced numbers indicate an increase in the degree of immune suppression (Bekker, 2010) which places the individual at risk for various infections or diseases. A category system for CD4 cell count allows health professionals to classify an individual infected with HIV/AIDS into one of three categories according to the CD4 cell count as pointed out in Table 4 (Weber, 2010).

Table 4: The three CD4 cell count categories

CD4 Cell Count Categories	CD4 cell count
Category 1	greater than or equal to 500 CD4+ T- cells/mm^3
Category 2	200-499 CD4+ T- cells/mm^3
Category 3	less than 200 CD4+ T- cells/mm^3

Adapted from CDC (1993)

A CD4 count is determined by means of a blood test that measures the number of CD4 cells in a sample of blood. It is a critical indicator of how the immune system is functioning. The CD4 count of a healthy adult range from 500 cells/mm^3 to 1 200 cells/mm^3. A very low CD4 count (less than 200 cells/mm^3) is one of the ways to establish if a person infected with HIV/AIDS has progressed to category 3 (Table 4).

People with category 3 are at significant risk of developing serious illnesses. A drop in the CD4 count indicates that HIV/AIDS is advancing and damaging the individual's immune

system. The CD4 count also helps to decide when to start ART. The higher the CD4 count, the better and more effective ART is to fight HIV and other infections (Bekker, 2010).

The progression from asymptomatic or acute HIV/AIDS (category A) to advanced HIV/AIDS (category C) is described and defined by the specific clinical conditions or symptoms (see Table 5) (CDC, 1993).

Table 5: CDC clinical categories of HIV/AIDS infection

Category	HIV/AIDS Related Symptoms, Diseases and Conditions
Category A	Asymptomatic HIV infection Persistent generalized lymphadenopathy Acute (primary) HIV infection with accompanying illness or history of acute HIV
Category B	Bacillary angiomatosis Candidiasis, oropharyngeal (thrush) Idiopathic thrombocytopenic purpura Listeriosis Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy Cervical dysplasia (moderate or severe)/cervical carcinoma in situ Constitutional symptoms, such as fever (38.5 C) or diarrhea lasting greater than 1 month Hairy leukoplakia, oral Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess Peripheral neuropathy
Category C	Candidiasis of bronchi, trachea, or lungs Candidiasis, esophageal Cervical cancer, invasive Cryptococcosis, extrapulmonary Coccidioidomycosis, disseminated or extrapulmonary Cryptosporidiosis, chronic intestinal (greater than 1 month's duration) Cytomegalovirus disease (other than liver, spleen, or nodes) Cytomegalovirus retinitis (with loss of vision) Encephalopathy, HIV-related Histoplasmosis, disseminated or extrapulmonary Kaposi's sarcoma Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis Isosporiasis, chronic intestinal (greater than 1 month's duration) Lymphoma, Burkitt's (or equivalent term) Lymphoma, immunoblastic (or equivalent term) Lymphoma, primary, of brain Mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary) Mycobacterium, other species or unidentified species, disseminated or extrapulmonary Pneumocystis carinii pneumonia Pneumonia, recurrent Progressive multifocal leukoencephalopathy Salmonella septicemia, recurrent Toxoplasmosis of brain Wasting syndrome due to HIV

Adapted from CDC (1993)

In Table 5 the CDC classifies the HIV/AIDS infection into three categories. These three clinical categories of HIV/AIDS infection are based on the clinical findings that guide the diagnosis, evaluation, and management of HIV/AIDS and it does not require a CD4 cell count. This clinical category system is used in some countries to determine if people with HIV/AIDS are eligible for antiretroviral therapy, particularly in settings in which CD4 testing are not available.

2.2.4. Treatment of HIV/AIDS

Due to the large number of people infected with HIV/AIDS in South Africa, the country has the world's largest ART programmes. Of the 21.2 million people in Africa eligible for ART in 2013 based on the 2013 WHO guidelines, only 7.6 million people (approximately 28%) were receiving HIV/AIDS treatment (Ref: UNAIDS Access to antiretroviral therapy in Africa: status report on progress towards the 2015 targets. Geneva, Switzerland: UNAIDS; 2016). In comparison, 48% of infected adults in South Africa receive ART treatment (UNAIDS, 2016). ART saves lives and prevents new HIV/AIDS infections (reduces risk of HIV transmission up to 96%), prevents illnesses (reduces risk of TB among people with HIV/AIDS by 65%), and keeps people productive in their work and environment. The treatment medication options available in South Africa are summarised in Table 6.

ART medication in South Africa has different types of fixed-dose combinations (FDCs) and it is increasingly being made available to people with HIV/AIDS. The oldest combination is zidovudine (AZT)/lamivudine (3TC), but a number of other two- and three-drug FDCs are now available in the region. These FDCs reduce the burden of multiple pills and may improve treatment adherence (Meintjes et al., 2015).

Table 6: The South African ART treatment medication

Drug name	Dosage
Abacavir (ABC)	300 mg/100 mg once daily or 600 mg/100 mg twice daily
Atazanavir + ritonavir (ATV/r)	300 mg/100 mg once daily
Darunavir + ritonavir (DRV/r)	600 mg/100 mg twice daily
Efavirenz (EFV) Swallow tablet whole	600 mg daily (or 400 mg if < 40 kg); usually given at night
Emtricitabine (FTC)	200 mg once daily
Etravirine (ETR)	200 mg twice daily
Lamivudine (3TC)	150 mg twice daily OR 300 mg once daily
Lopinavir + ritonavir (LPV/r) Swallow tablet whole	400 mg/100 mg twice daily NB: Patients on a rifampicin-containing TB regimen must have their dose increased to LPV/r 800/200 mg twice daily
Nevirapine (NVP)	200 mg daily for 2 weeks*, then 200 mg twice daily
Raltegravir (RAL)	400 mg twice daily
Stavudine (d4T)	30 mg twice daily
Tenofovir (TDF)	300 mg once daily
Zidovudine (AZT)	300 mg twice daily

Meintjes, Black, Conradie, Dlamini, Maartens, Manzini, Mathe, Moorhause, Moosa, Nash and Oral (2015)

ART have a significant positive effect on the life expectancy of people infected with HIV/AIDS, these therapies should not be regarded as a cure for HIV/AIDS (Swanepoel & Louw, 2010). The ART medications are important and necessary for many people with HIV/AIDS, but there are side effects associated with the use of these drugs. Certain auditory disorders can be caused by the antiretroviral treatment. Ototoxic medications can affect the inner ear, causing a sensorineural hearing loss. Constant auditory monitoring of people taking ototoxic medication is essential for the early identification of potential ototoxicity and the consequent development of a severe hearing loss. The actual association between ART therapies and ototoxicity is not entirely clear and therefore further research is necessary to establish the real cause of hearing loss in people infected with HIV/AIDS in all stages, including people who have HIV/AIDS but are not using any medication (Stearn & Swanepoel, 2010).

2.3. Anatomy, physiology, and pathologies of the auditory system

The human auditory system is divided into two parts, namely the **peripheral hearing mechanism** (external ear, middle ear and inner ear) (Figure 3) and the **central hearing mechanism** (from the cochlear nucleus up to the primary auditory cortex). Studies have shown that the progression of HIV/AIDS may impair the central nervous system (CNS) and modify the auditory system in both its peripheral and central areas, thereby justifying the investigation of the auditory pathway as a whole (Quidicomo & Matas, 2013).

2.3.1. Central hearing mechanism

The auditory sensorineural system consists of the cochlea and the eighth cranial nerve and it prompts a physiological response to the stimulus that activates the nerve cells and encodes the sensory response into a neural signal. The central auditory nervous system then distributes this encoded signal further (Gelfand, 2009) see Figure 2.

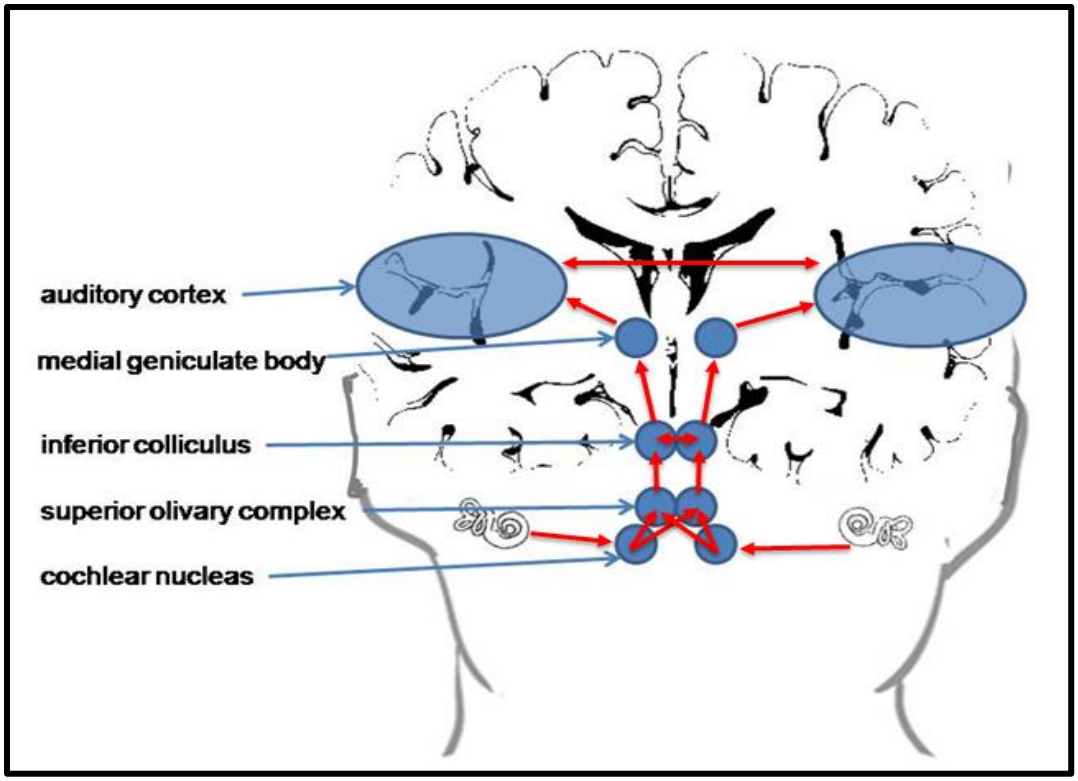


Figure 2: Horizontal section of the auditory cortex

- The **auditory nerve** exits the temporal bone through the internal auditory meatus and enters the brainstem at the cerebellopontine angle. The auditory nerve fibres end at the ventral cochlear nucleus or the dorsal cochlear nucleus, where it synapses with the next level of nerve cells. Each superior olivary complex receives sounds from both ears. The neurons that emerge from the superior olivary complex, as well as the fibres that originate from the lateral lemniscus (originated from different origins), are situated alongside the lateral lemniscus. Neurons will synapse or pass the inferior colliculus. All the neurons will terminate at the medial geniculate body (Gelfand, 2009).

- The **cochlear nucleus (CN)** is the most caudal structure in the CANS (Bellis, 2008), and is the first level of the subcortical auditory process that is mediated at this level (Hackett, 2008). It forms the origin of basic response patterns and the emergence of parallel pathways (Winer & Schreiner, 2005). The cochlear nerve and the 8th auditory nerve are projected together to the section of the cochlear nucleus and diverge when it meets the higher regions of the brainstem (Hackett, 2008). The principal function of the CN is to extract, enhance, and integrate the auditory input (Bellis, 2008).

- The **superior olivary complex (SOC)** is the second level of auditory brainstem processing. It obtains bilateral projections from the cochlear nuclei (Hackett, 2004). It is an important component of the binaural (ascending and descending) auditory pathways of the auditory system. The medial superior olivary complex measures the time difference of arrival of sounds between the ears and it forms time lines (Winer & Schreiner, 2005). It is also the first initial stage of central auditory processing where the inputs from both ears integrate (Hackett, 2008). It is also important for auditory stimuli localisation and necessary for hearing in the presence of background noise (Bellis, 2008).

- The **lateral lemniscus (LL)** is the primary fibre tract that links the superior olivary complex with the IC (Hackett, 2008). This is where the development of the chemically specific nuclei originates (Winer & Schreiner, 2005). It is also part of the primary ascending auditory pathway (Bellis, 2008).

- The **inferior colliculus (IC)** is the location where brainstem convergence and multisensory integration occurs (Winer & Schreiner, 2005). This is the largest auditory structure of the brainstem and it is sensitive to binaural stimulation (Debonis & Donohue, 2004). Several ascending and descending auditory pathways converge in the IC from the auditory nuclei bilateral of the brainstem. Descending inputs from the superior colliculus, thalamus, and cortex also converge into the IC (Hackett, 2008). The IC identifies sound sources and processes other binaural cues (Bellis, 2008).

- The **medial geniculate body (MGB)** is the last level of subcortical processing of the ascending auditory information. The ascending inputs arise from all parts of the IC bilaterally. The outputs go directly to the primary and non-primary fields of the auditory cortex and then back to the medial geniculate body, forming a feedback loop (Hackett, 2008). This is where the detection of the relative intensity and duration of a sound takes place. The MGB shows a wide range of responses to auditory stimuli. Strong modulatory influences from cortical feedback and many extra cortical sources have been described. Its function may be related to modulation and gating of activity forwarded to the cortex (Winer & Schreiner, 2005). The MGB is situated in the thalamus (Brugge, 2013). Speech signals for example vowels and slow changing consonant stimuli encode successfully at this level (Bellis, 2008).

The physiology of the auditory pathway is set into motion when an auditory signal is received and passes through the eighth cranial nerve (auditory nerve) to the brainstem. The auditory signal moves back and forth through the neural fibres which link the brainstem to the cerebral cortex (temporal lobe). Information from the right ear passes on directly to the left hemisphere. The information presented to the left ear travels to the right hemisphere via the corpus callosum (Bellis, 2008). Dichotic (binaural) hearing (see Figure 3) occurs when auditory stimuli are presented to both ears simultaneously (Bellis, 2008). The perception and verbal labelling of an auditory linguistic stimulus requires processing by both hemispheres of the brain. The left hemisphere is typically language dominant and is required for the verbal response. Figure 3 indicates that information presented to both ears (dichotic listening) is, on the one hand, subject to the integrity of the left hemisphere. On the other hand, if the right hemisphere or corpus callosum has a dysfunction, the left ear will be affected (Bellis, 2008).

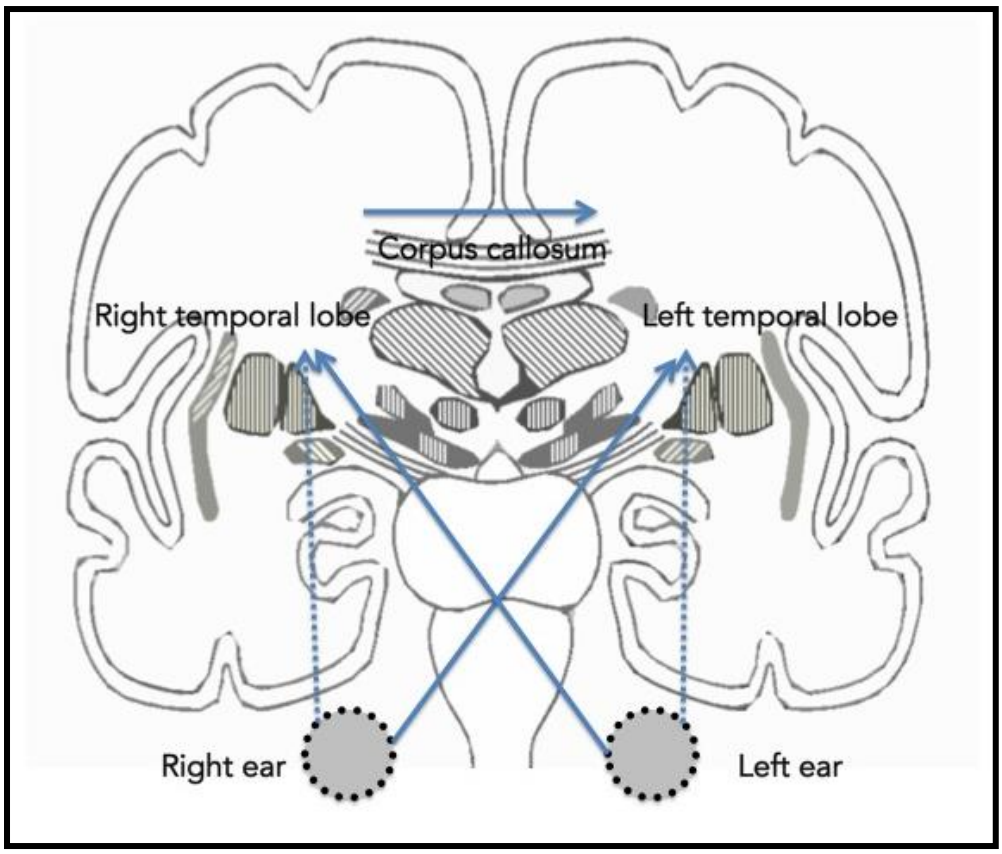


Figure 3: Dichotic listening (Adapted from Bellis, 2008)

The **auditory cortex** is responsible for the processing of auditory stimuli and consists of the superior half of the temporal lobe and is divided into three areas namely core, belt (surrounding the core area), and parabelt (Hackett, 2008). The areas of auditory reception are located in the temporal lobe where Heschl's gyri are situated (Debonis & Donohue, 2004). The auditory cortex is responsible for auditory perception, communication, and cognition (Winer & Schreiner, 2005). The primary auditory cortex consists of Brodmann area 41 and Brodmann area 42 which form part of the auditory association area (Wernicke's area). The auditory areas (primary auditory cortex (PAC), BA 41 and BA 42) are found in the lateral surface of the cortex of the superior temporal gyri and deep in the lateral sylvian fissure (Brugge, 2013). The auditory cortex can be subdivided in areas according to the neurophysiology of the central auditory nervous system (Figure 5). It is an important part in the brain for the supply of acoustic information from the lower levels including the cochlea to the higher levels in the temporal lobe of the cortex at Heschl's gyrus. A significant characteristic is the presence of neural redundancies displayed in multiple afferent (ascending) and efferent (descending) pathways with several crossover points (Richard, 2001).

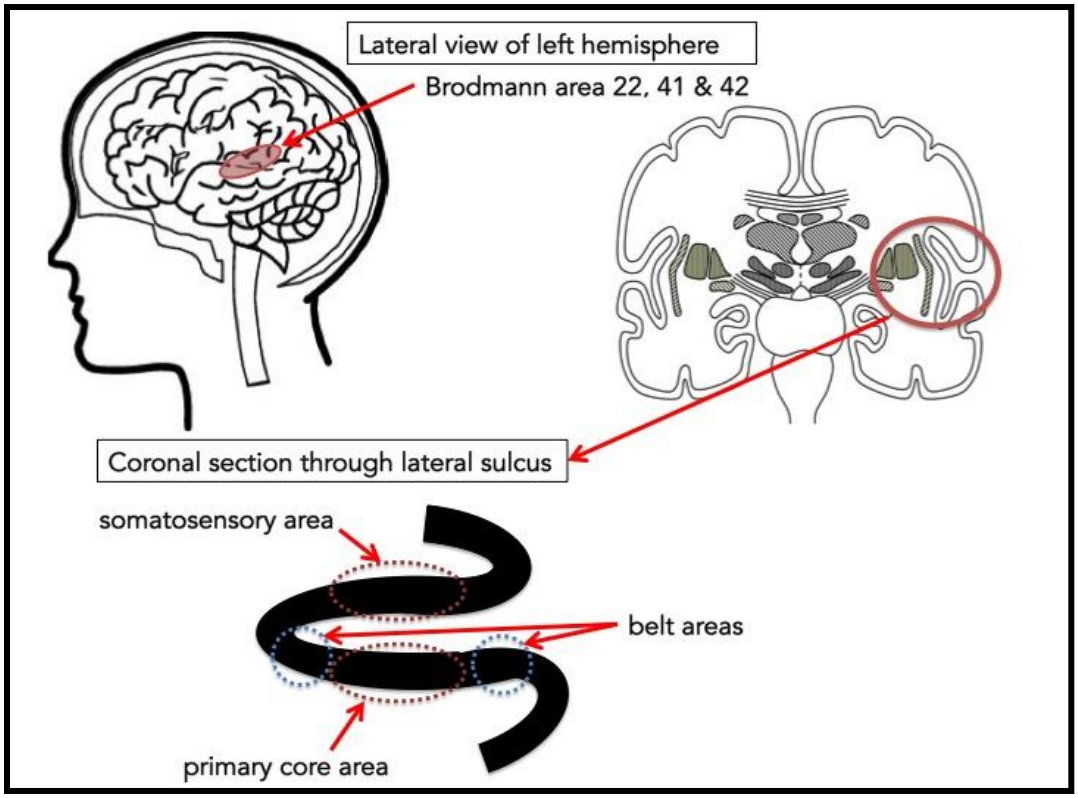


Figure 4: Lateral view of the left hemisphere and horisontal section of the auditory cortex (Adapted from Hackett, 2008)

Figure 4 shows the lateral view of the left hemisphere where Brodmann 22, 41 and 42 are situated. These areas are prominent in auditory and language processing (Richard, 2001). The different levels of subcortical auditory processing, as described in Figure 4, are discussed in detail below.

- **Brodmann (BA) 22** **Auditory association cortex**

  The cortex of the transverse gyrus of Heschl forms a homogeneous structural region. This *superior temporal area* intrudes the posterior two-thirds of the superior temporal gyrus and is partially occupied by Brodmann areas 41 and 42 (Brodmann, 1909). BA 22, also known as Wernicke's area, recognises linguistic (verbal) stimuli and it is responsible for the comprehension of spoken language and language formation (Bellis, 2008).

- **Brodmann (BA) 41** **Primary auditory cortex**

  This *medial (anterior) transverse temporal area* links to the anterior transverse gyrus and extends obliquely from anterolateral to posteromedial. It descends gradually into the depths of the sulcus. It is bordered medially by the parainsular area (Brodmann, 1909).

- **Brodmann (BA) 42** **Secondary auditory cortex**

  This *lateral (posterior) transverse temporal area* ranges obliquely from anterolateral to posteromedial over the superior area of the superior temporal gyrus. It takes the form of a crescent along the lateral edge of area 41. Caudally it extends deeply towards the posterior edge of the insula (Brodmann, 1909).

The left hemisphere is larger than the right hemisphere. Heschl's gyri (occupying BA 41 and BA 42) are seven times larger in the left hemisphere than in the right hemisphere. The lateral sulcus divides both the frontal and parietal lobe. The lateral sulci are situated in both hemispheres of the brain, but the sulcus is longer in the left hemisphere than in the right hemisphere and the post-temporal area is larger in the left than the right (Richard, 2001). A coronal section through the lateral sulcus shows the different areas such as the somatosensory area, belt areas (surround the core area), and primary core area (Hackett, 2008). Auditory processing includes numerous parallel pathways. Acoustic information circulates from the top in the auditory cortex to the lower part of the auditory system where the cochlea is situated. The auditory information in the auditory cortex is processed, as needed, by other centers of the brain, such as various language-processing centers, to make sense of the incoming sensory information (Hackett, 2008).

Normal peripheral hearing is essential for the processing of any auditory stimuli. Figure 5 is a schematic representation of the processing continuum model from the peripheral auditory system up to the auditory cortex.

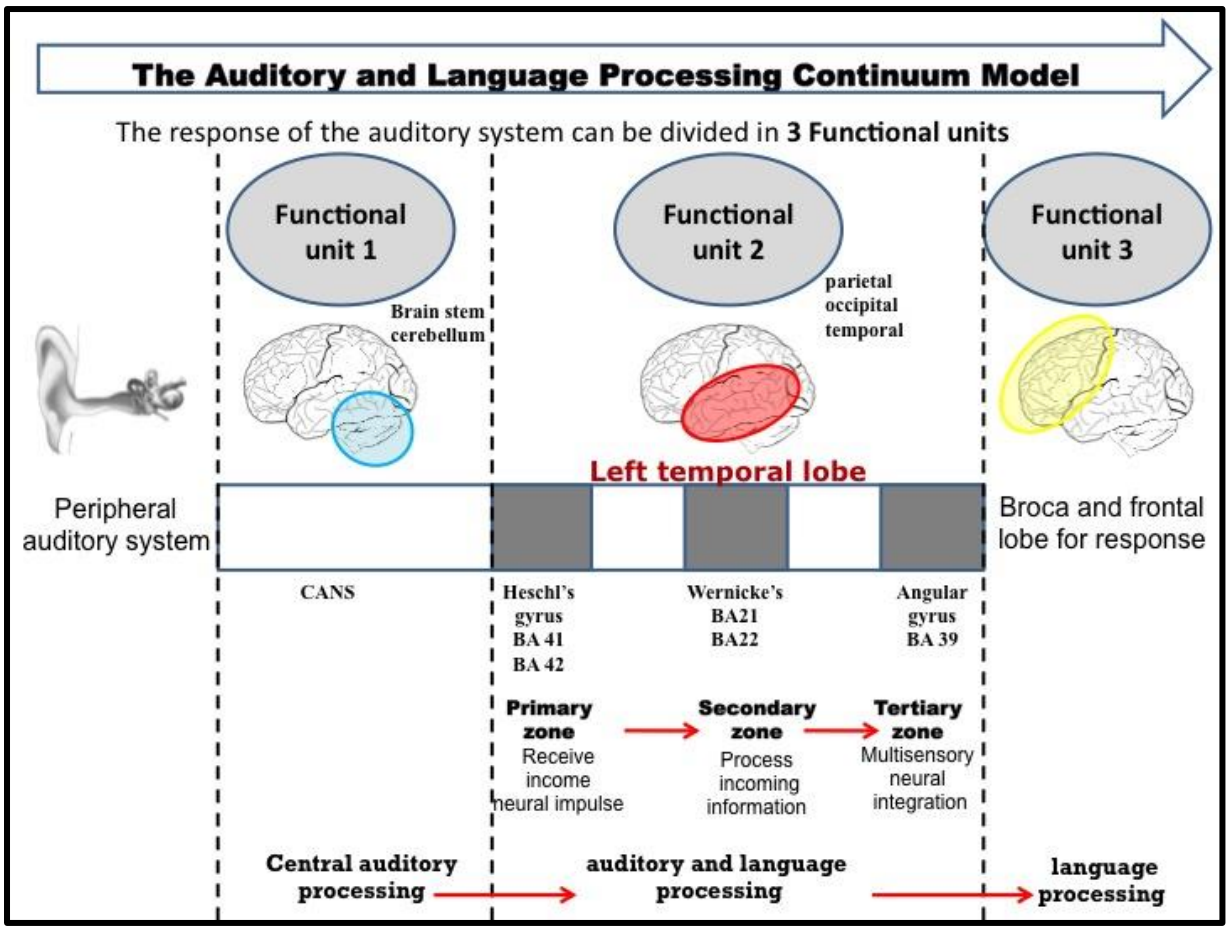


Figure 5: Sketch of the auditory processing continuum (Adapted from Richard, 2001)

Central auditory processing implicates the neurological pathway of the auditory signal as it enters the cortex at Heschl's gyrus. The primary auditory cortex is located in the medial part of Heschl's gyrus that processes speech and language information received from the auditory signal. Figure 5 indicates that when speech is heard, it enters the left temporal lobe through Heschl's gyrus and it is at this point that auditory processing takes place. Acoustic features are processed and interpreted by the listener at this level to derive meaning (language processing). The continuum model also described the brain areas that are responsible for the processing of auditory stimuli (Richard, 2001). The left temporal lobe in Figure 5 is part of the auditory and language processing areas at the level of the left temporal lobe. The function of the left temporal lobe is summarised in Table 7 in terms of different zones namely the primary, secondary, and tertiary zone.

Table 7: The function of the left temporal lobe in the language processing model

	Primary zone	Secondary zone	Tertiary zone
Function	Auditory sensation	Attach meaning	Integrate auditory information
Purpose	Auditory integration	Language integration	Cortical integration
Brodmann area	41, 42	21, 22	39
Anatomical structure	Heschl's gyrus	Wernick's area	Angular gyrus
Summary	Auditory input	Processing	Integration to output

Adapted from Richard (2001)

As illustrated in Figure 5 and summarised in Table 7 sensory stimuli enter the cortex from the brainstem and then continue to the left temporal lobe, where the primary zone is known as the Brodmann area 41 and the Brodmann area 42 (part of Heschl's gyri). In the left temporal lobe, the association areas are known as Brodmann area 21 and Brodmann area 22 (Wernicke's area, which also forms part of the language area). There is a tertiary zone in each lobe which is a communal area where the intersection of the secondary zones occurs. Brodmann area 39 (angular gyrus) is the integration area for auditory information (Richard, 2001).

In Figure 5 and Figure 6 the functional organisation of the brain is described. The central nervous system is divided into three functional units namely **functional unit 1, functional unit 2** and **functional unit 3.**

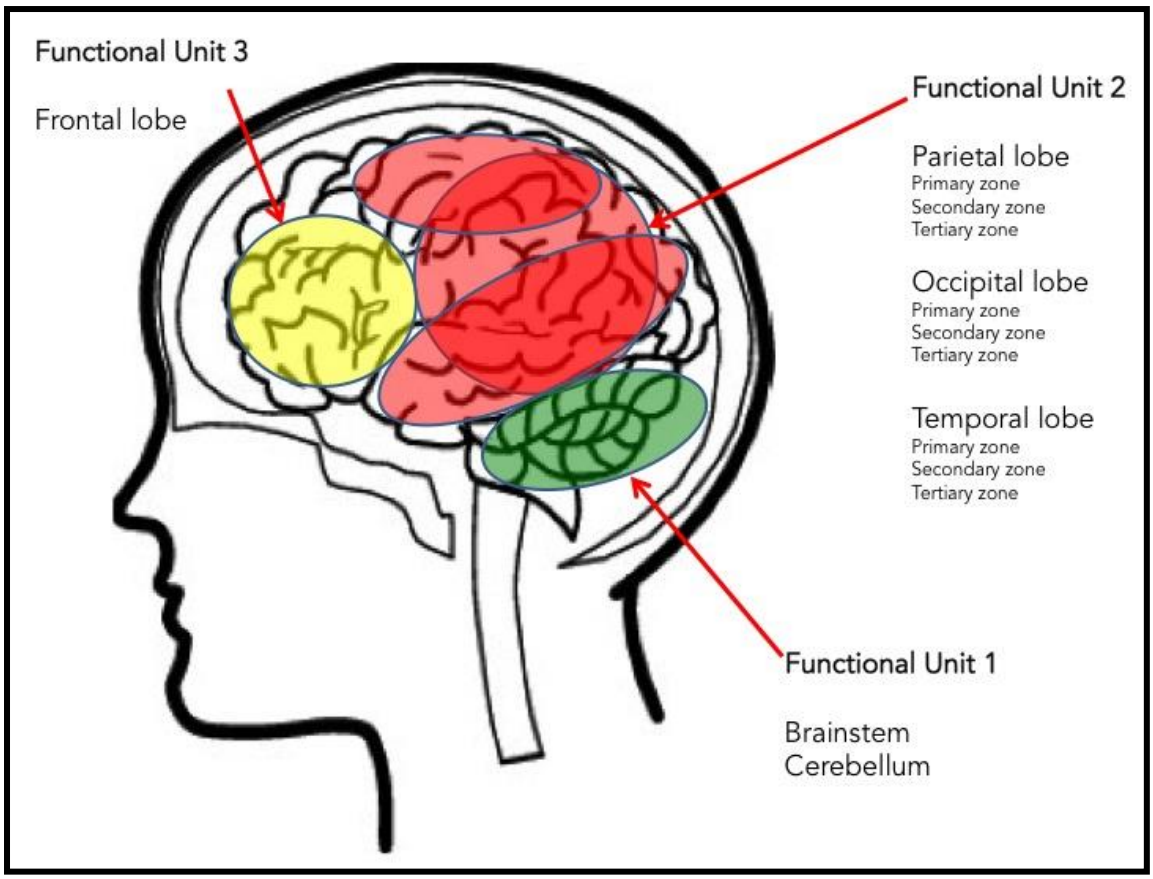


Figure 6: Functional organisation of the brain (Adapted from Richard, 2001)

Functional unit 1 (see Figure 6) is the reticular formation and it consists of the *brainstem* and the *cerebellum* that form part of the CANS. It is an important unit for processing and it sends the stimuli to the cortex area of the brain (Richard, 2001).

Functional unit 2 (see Figure 6) consists of the *parietal*, *occipital*, and *temporal lobes*. It is important for the neural impulses to be divided into separate regions for analysis, storage, coding and organisation. Visual stimuli are processed in the occipital lobes, tactile stimuli are processed in the parietal lobes, and auditory stimuli are processed in the temporal lobe (Richard, 2001). The *parietal lobe* is partly responsible for the perception and elaboration of somatic sensation for the distribution of attention. The *temporal lobe* is part of the primary auditory cortex and the auditory association areas including Wernicke's area (BA 22) and the hippocampal formation that is partly responsible for the processing of emotion and memory. The primary and secondary visual cortices are located in the *occipital lobe* (Bellis, 2008).

Functional unit 2 is responsible for the auditory and language processing that takes place in the cortex, and it is also divided into three zones namely ***primary***, ***secondary*** and ***tertiary*** zones.

The ***primary zone*** receives auditory impulses. Visual information arrives at the cortex in the primary zone of the occipital lobe, tactile stimuli enter the primary zone of the parietal lobe, and auditory information enters the cortex in the primary zone of the temporal lobe. Therefore impairment of the primary zone will be a sensory impairment (impaired cortical hearing) (Richard, 2001).

The ***secondary zone*** is involved with the processing of the incoming information and attaches meaning to the sensory input that is received in the primary zone. Stimuli are combined into meaningful experiences in the secondary zone of the specific cortex. Visual processing takes place in the secondary zone of occipital lobe, tactile processing takes place in the secondary zone of the parietal lobe, and auditory processing in the secondary zone of temporal lobe reveals the meaning of auditory stimuli (Richard, 2001).

The ***tertiary zone*** is part of the higher level of processing and enhances the auditory processing that took place in the secondary zone (Richard, 2001).

Functional unit 3 (see Figure 6) consists of the *frontal lobes*. Its response is by means of a motoric processing to the stimuli that were processed in the second functional unit. Perception and knowledge are facilitated in this third functional unit (Richard, 2001). It is also part of higher order planning, and is partly responsible for the execution of behaviour and for motoric actions of the entire body (Bellis, 2008).

From the above HIV/AIDS can infect the brain and affect the central nervous system (CNS) function. A combination of ART has had a major impact on all aspects of HIV-1 CNS infection and disease, but the possibility of complications has not been eliminated. Cognitive impairment in HIV/AIDS disease remains common (Valcour, Sithinamsuwan, Letendre & Ances, 2010). HIV-associated neurocognitive disorder (HAND) is the result of neural damage caused by HIV replication and immune activation. Patients should be continually monitored, as cognitive impairment might persist or present for the first time during antiretroviral therapy (Valcour et al., 2011).

2.4. HIV/AIDS in the peripheral auditory system and central auditory system

Studies have shown that the progression of HIV/AIDS may impair the CNS and affect the auditory system in both the peripheral and central areas, justifying the investigation of the auditory pathway as a whole (Quidicomo & Matas, 2013). The possible influence of HIV on the various parts of the hearing mechanism is described below.

- **External ear (outer ear)**

The pinna and the external auditory canal are prone to infectious and dermatological conditions related to HIV (Gold & Tami, 1998).

- **Middle ear**

People with HIV more often have Eustachian Tube dysfunction due to recurrent acute sinusitis that is common in people with HIV. The infected mucus causes swelling around the Eustachian Tube and this can cause dysfunction of the Eustachian Tube (Swanepoel & Louw, 2010). Aural polyps may be present in the middle ear, but can also extend into the ear canal due to mycobacterium tuberculosis infection. Clear otorrhea will be observed. Otitis media with suppurative middle ear effusion often occurs in adults with HIV (Gold & Tami, 1998). In both of these conditions, a conductive hearing loss will be experienced.

- **Inner ear**

The inner ear houses the sensory organs for hearing (cochlear system) and balance (vestibular system). HIV/AIDS tends to cause demyelisation of the neurons in the CNS and this can affect the vestibulo-cochlear nerve (Gold & Tami, 1998). If HIV/AIDS has affected the central part of the hearing system, the direct effect of the virus on the cochlea and the auditory nerve could have an additive effect (Assuiti et al., 2013) An ultrastructural study (Poppas, Sekhar, Lim, & Hillman, 1994) of the cochlea of patients with HIV/AIDS showed pathological changes in the labyrinth wall, the epithelial lining and the maculae and cristae. Viral-like particles were also detected in the auditory and vestibular hair cells, strial cells, and along the tectorial membranes of people with HIV/AIDS. This evidence of viral-like particles

and cochlear pathology provides an insight into the likely pathogenesis of viral-induced hearing loss and vestibular impairment in patients infected with HIV/AIDS (Poppas et al., 1994). Such inner ear impairments and cochlear pathology may cause a sensorineural hearing loss related directly linked to HIV/AIDS virus and ART drugs. That this disease and its associated risk profile have direct effects on the vestibular system is demonstrated by post-mortem studies. Opportunistic infections may also compromise the functioning of the sensory and neural structures of hearing and the vestibular system indirectly, causing vertigo, dizziness, or disequilibrium. Vestibular dysfunction may also be caused / related to vestibular dysfunction due to the ototoxic nature of certain ART medications (Matas et al., 2014).

- **Central auditory nervous system (CANS)**

The largest outcome of HIV/AIDS infection on the auditory system appears in the form of abnormalities in the CANS (Maro et al., 2014). The infection or inflammation can cause several neurocognitive side effects and has the potential to affect the eighth cranial nerve to such an extent that sensorineural hearing loss can occur in about 75 % of the adults infected (Assuiti et al., 2013, Maro et al., 2014). Grimaldi, Luzi, Martino, Furlan, Nemni, Antonelli, Canal and Pozza (1993) explained that bilateral vestibulocochlear nerve neuropathy can develop in patients with HIV/AIDS and cause sudden bilateral hearing loss.

The effect of HIV/AIDS on the central auditory system can be direct as well as indirect. Firstly, the direct effect of HIV/AIDS on the central nervous system may cause a disintegration and demyelination of the auditory pathways. Secondly, it may also cause infections that can lead to sensorineural hearing loss. Thirdly, the ototoxicity from antiretroviral medication for HIV/AIDS and medications used for secondary infections caused by HIV/AIDS, are also a risk that can lead to hearing loss (Stearn & Swanepoel, 2010).

HIV/AIDS virus itself can have various effects on the central nervous system, such as encephalopathy, subacute encephalitis, cryptococcal meningitis, central nervous system toxoplasmosis, tuberculous meningitis, bacterial and viral meningitis, all of which may lead to sensorineural hearing loss

Any disorder in the CANS affects the system's ability to use auditory stimuli effectively and efficiently. It may have a profound influence on the individual's ability to listen, learn, and navigate through social environments (Elsisy, 2013). These pathways are quite complex and if a lesion should occur at any point within the pathway, it will rarely cause a loss of sensitivity because the signal will flow to another pathway. Significant disturbances can, however, be caused by a central lesion, because it can affect the processing of the auditory information (Gelfand, 2009).

Over the last few years various studies have been done to determine the effects of HIV/AIDS on the auditory system. A summary of procedures that have been utilised to evaluate central or peripheral auditory ability in people with HIV/AIDS and the related findings are presented in Table 8.

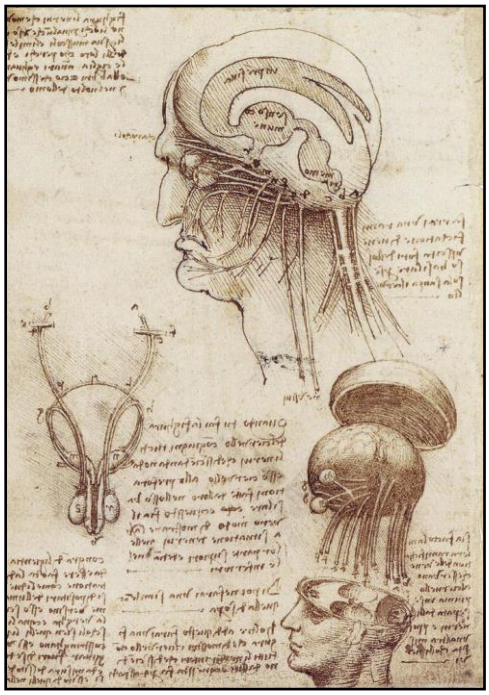
Table 8: HIV/AIDS and the central and/or peripheral auditory systems: Procedures and findings

Author year of publication	Procedures	Primary Findings	
		Peripheral	Central
Torre III et al., (2016)	Otoscopy Immittance Testing	HIV/AIDS has an effect on the middle ear function causing middle ear infection and otorrhea	
Romero, Alfaya, Gonçales, Frizzo & de Lima Isaac (2017)	Pure tone audiometry Auditory Processing Test	HIV/AIDS causes a high incidence of auditory peripheral impairment	HIV/AIDS cause a high incidence of auditory central impairment
Torre (2015)	Auditory brainstem response *(*ABR) Distortion product otoacoustic emissions (DPOAE)		HIV/AIDS people have of poorer quality of hearing results than HIV/AIDS uninfected participants, HIV/AIDS related central nervous system disorders affect the central auditory system
Fokouo, Vokwely, Noubiap, Nouthe, Zafack, Ngom, Dalil, Nyeki, Bengono & Njock (2015)	Pure tone audiometry	HIV/AIDS causes otologic problems such as vertigo, tinnitus, otalgia. Hearing loss associated with HIV/AIDS (sensorineural (61.7%), mixed (20%), conductive (18.3%)	
Matas, Samelli, Angrisani, Magliaro & Segurado (2015)	Pure tone audiometry Brainstem auditory evoked potential (BAEP)	PTA indicates middle ear pathology in participants with HIV/AIDS	Participants with HIV/AIDS have higher percentage of altered AEBR responses that suggests central auditory pathway impairment. Impairment in the auditory pathway at the brainstem level due to direct action of HIV/AIDS.
Matas et al., (2014)	Pure tone audiometry Speech audiometry High frequency audiometry	Participants with HIV/AIDS had significantly poorer results with pure tone audiometry and speech audiometry, possibly due to the impairment of the peripheral auditory pathway and high frequency audiometry	
Maro et al., (2014)	Pure tone audiometry Otoacoustic emissions (OAE) Immittance Testing	Pure tone audiometry & Immittance testing had similar results for participants with HIV/AIDS and participants without HIV/AIDS. HIV/AIDS participants had reduced OAE's. Participants on ART had greater difficulties understanding speech-in-noise.	
Pluta, Kurkowski, Rusiniak, Wolak, Wasilewska, Grudzien & Skarzynski (2011)	fMRI		(APD) was evident in the HIV/AIDS groups due to smaller and less activation in the brain areas: superior frontal gyrus/superior motor area bilaterally, left anterior insula, left parietal lobule. These areas implicated problems in behavioural and language processing.
Buriti et al., (2013)	Immittance Testing	Higher incidence of Type B tympanograms indicated middle ear pathology	
Quidicomo et al., (2013)	Pure tone audiometry Speech audiometry High frequency audiometry	Participants with HIV/AIDS presented with more audiologic pathology, inner ear impairments and with higher occurrence of sensorineural hearing loss	
Luque, Orlando, Leong, Allen, Guido, Yang & Wu (2014)	Pure tone audiometry Immittance Testing	Elevated pure tone thresholds, especially in the low frequencies in participants with HIV/AIDS	

From the table (Table 8) it is clear that most of the studies focussed on the influence of HIV/AIDS on the peripheral auditory system using standard audiological procedures (pure tone audiometry, immittance testing, otoacoustic emissions) and to a lesser degree on the central system using electrophysiological testing (ABR) and more specific tests such as tests for auditory processing and fMRI. It is evident that HIV/AIDS play a role in various areas of the auditory system. According to Torre (2015) the differences in auditory sensitivity found based on HIV/AIDS status might be the result of the influence thereof on auditory neural function. However, during early stages of HIV/AIDS infection when no clinical manifestations (normal pure tone thresholds) are present, abnormalities might still occur, as determined by ABR recordings.

Research in this regard is thus crucial in order to determine the influence of HIV/AIDS on the auditory system even before the condition progresses to a symptomatic stage. The use of fMRI with blood oxygen level-dependent (BOLD) contrast will highlight the areas in which either no neural activity or abnormal activity might be indicative of an underlying auditory processing problem.

2.5. Assessment of CANS using fMRI



Physicians of the renaissance and post-renaissance periods produced theories about brain functions, but were unable to verify their theories. In the 19th century professionals thought of new ways or ideas to investigate or research the brain and its functioning and identified different areas of the brain. Figure 7 represents Leonardo da Vinci's "study of brain physiology", where he suggested different functions in the ventricular system. These sketches can be considered the first attempt to visualize brain functioning.

Figure 7: Study of Brain Physiology, c.1508 (Heuttel et al., 2009)

More than 500 years later (21st century), scientists were able to obtain a far more accurate picture of the brain's functioning by using functional magnetic resonance imaging (fMRI), a neuroimaging technique that uses an MRI scanner to examine the brain function over a period of time (Heuttel et al., 2009).

Magnetic resonance imaging involves the interaction of matter with electromagnetic fields. A proton constantly turns and spins around an axis as in Figure 8. The proton forms a positive electrical charge (electrical current) that induces a magnetic field. The human body is largely composed of water molecules, each containing two hydrogen nuclei, or protons. When inside the magnetic field (B_0) of the scanner, the magnetic moments of these protons align with the direction of the field.

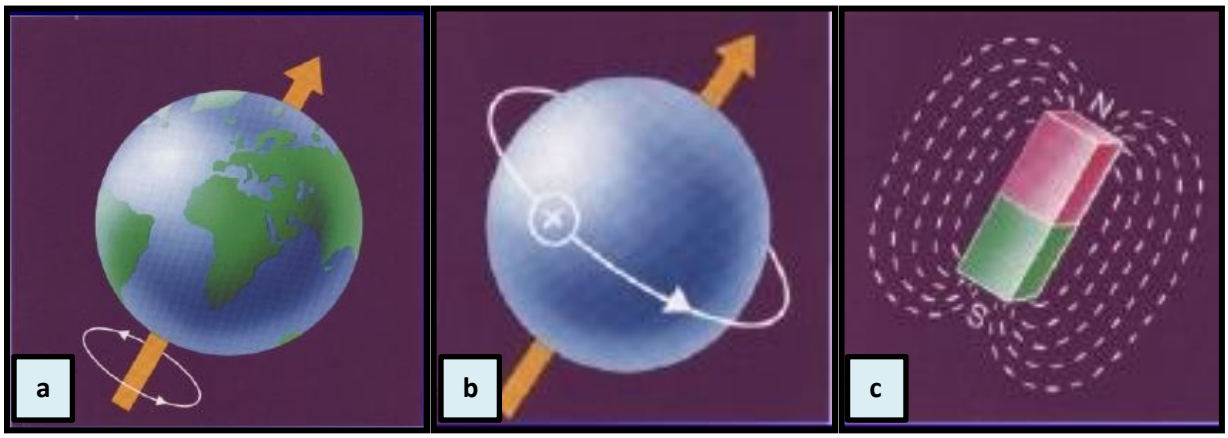


Figure 8: (a) A proton is like earth, (b) it forms a positive electrical charge when spinning and (c) induces a magnetic field (Hans, 1990)

The protons (positive charge) in a magnetic field spin around their own axis and this movement is called precession. The speed of the protons is called "precession frequency", and it refers to the number of times the protons precess per second. A larmor equation is used to calculate the frequency (Hans, 1990).

The larmor equation $$\omega_o = \gamma B_0$$

ω_0 = precession frequency (Hz)
B_0 = strength of external magnetic field (T)
γ = gyro magnetic ration

This calculation shows that an increase of the precession frequency will only occur if the magnetic field strength increases. A new magnetic vector is induced into the patient when the patient is in a strong magnetic field. The patient then becomes a magnet himself. This magnetic vector in the patient is subsequently aligned with the outside magnetic field. This new magnetic vector is used to obtain a signal to generate images (Hans, 1990).

A pulse sequence is needed to create an MR image. The sequence will include radiofrequency (RF) pulses and gradient pulses that are controlled by duration and timing. The gradient pulses make a typical "knocking" noise during the scan. There are different kinds of sequences with timing values namely "TR" and "TE". These can be controlled by the operator. MR is grounded on the usual magnetisation that is provoked in the human body when it is positioned inside the scanner. This is the basis of MRI (McRobbie, Moore, Graves & Prince, 2017).

2.5.1. Magnetic resonance imaging

MRI uses magnetic fields created as explained above in order to produce images (Heuttel et al., 2008). The first MRI scanner was built in 1977 and functional MR imaging of the brain was introduced in1993 (Ashby, 2011). The MRI scanner has a static magnetic field in the middle and it does not change over time. The strength of the magnetic field itself is measured in Tesla (T). Scanners used for fMRI are usually 1.5 T, but it can also be 3T. A pulse sequence is a series of fluctuating electromagnetic fields and magnetic gradients. The frequency (tuned to the frequency of hydrogen nuclei which are abundant in the human body) will be absorbed by atomic nuclei and the electromagnetic energy will then be emitted by the nuclei. The differentiation between tissue types depends on the pulse sequence used. Structural studies using MRI of the brain have been used to identify or investigate neurological disorders. Structural studies are restricted to investigate the active functioning of the brain (Heuttel et al., 2009). Functional magnetic resonance imaging (fMRI) is a functional neuroimaging procedure using MRI technology that measures brain activity by detecting associated changes in blood flow.

2.5.2. Functional Magnetic Resonance Imaging (fMRI)

In 1936 Linus Pauling learned that a hemoglobin molecule has magnetic properties when it binds to oxygen. The MRI sequence will show a clearer signal if the blood is highly oxygenated (enriched with oxygen) and will show a reduced signal if it is highly deoxygenated (oxygen removed). The work of Ogawa and his colleagues in 1990, showed that MRI can be used to measure the changes in blood oxygenation. BOLD (blood oxygenation level dependent) fMRI measures changes in the complete quantity of deoxygenated hemoglobin (Heuttel et al., 2009). The concept is that when the brain is active in a certain area it uses more oxygen than in the nonactive areas (Ashby, 2011).

Functional Magnetic Resonance Imaging (fMRI) measures the changes in blood oxygenation over time and it is done in a non-invasive manner. Structural studies of the brain have become quite easy to analyse, but it is limited to showing short-term physiological changes that are related to the functioning of the brain. When an area of the brain is in use, blood flow to that region also increases (Heuttel et al., 2009). Physicians use fMRI to assess brain functioning before surgery (invasive treatment) to learn how a normal, diseased, or injured brain is functioning.

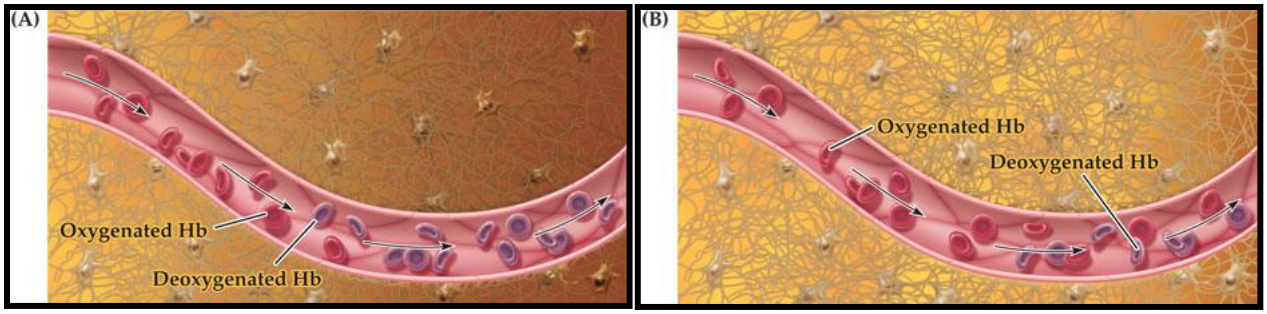


Figure 9: BOLD signal generation (Huettel et al., 2009)

Figure 9 (A) illustrates a normal environment where oxygenated hemoglobin (Hb) is at a steady rate with a capillary layer. Figure 9 (B) indicates that when there is an increase of blood flow, the neurons become active and the vascular structure supplies more oxygenated hemoglobin than is needed by the neurons. An increase of blood flow is needed to make the MR image brighter (Heuttel et al., 2009).

In fMRI there are two types of experimental designs, namely, a blocked design and an event related design. A blocked design consists of two or more conditions in an interchanging pattern and each block can be 10 to 30 seconds in duration. An event related design is a short duration of events during which timing and order may be random (Heuttel et al., 2009).

Two types of scans are done in the imaging session, namely structural MRI and functional MRI. Structural MRI shows more detail and soft tissue of the brain. Functional MRI shows changes in the brain at a specific time and fMRI data are then gathered as a time series (Heuttel et al., 2009). MRI studies normally use 12 to 20 subjects in an experiment. One experimental session can be between one and two hours for each session. Structural anatomy data with the same slice prescription as the fMRI is also obtained, and is used to align the functional data to a high-resolution reference volume. A session can be divided into several runs. In each run of the functional data a time series of volumes is obtained. Each volume consists of a number of slices depending on the area covered. Each slice consists of voxels, which then form an image of the brain that can be used for analysing (Heuttel et al., 2009).

Pre-processing of fMRI data starts before the statistical analysis of data can begin, and there are a few steps that need to be completed. The two main objectives are to reverse the movement of data in time and to improve the ability to detect spatially prolonged signals within or across subjects (Aguirre, 2011). During a scan it is imperative to reduce head motion. The head can be padded with foam, but even with padding the subject may still move his/her head during the scan. To correct such a motion, a data pre-processing step is necessary namely motion correction. This step realigns the image of the brain acquired at every point in time back to the first image obtained when the scanning started (Aguirre, 2011). Spatial smoothing is done with a filter to smooth the data. This filter can be the same size (voxels) as the active region of interests (Aguirre, 2011).

Statistical analysis of the BOLD fMRI data can be performed using a univariate or multivariate technique. In general the univariate technique is used. In the data set the statistical model is applied to each voxel, and this is done automatically in the software package used. The outcome of the statistical model will consist of covariates of interest and no interest. These covariates (beta values) are used to assess the time series data of each voxel in the brain. The last step of analysis is to allocate a level of statistical significance to those values.

The hypothesis test will be better if using a described anatomical region of interest areas. This will decrease the number of independent statistical tests that requires correction to improve power (Aguirre, 2011).

The general linear model consists of four matrix designs that represent the data that were obtained from the experiment. The experimenter that established the hypothesised effects of the experimental manipulations, parameter weights, and residual error calculated during the analysis, forms the design matrix. The general linear model (GLM) is used by most fMRI statistical packages as it offers a theoretical framework (Heuttel et al., 2009).

$$\boldsymbol{Y = X * \beta + \varepsilon}$$

- **Data matrix:** fMRI data (Y) correspond with two dimensional data matrix BOLD signals at various time points (n rows) by voxels (V columns)
- **Design matrix:** The design matrix (G) specifies the linear model assessed, and consists of (M columns) regressors at time points (n rows) in length.
- **Parameter matrix:** Voxels (V rows) by (M columns) parameter weights.
- **Error matrix:** Express the residual error for each voxel and therefore it is time points (n rows) by voxels (V columns).

Software packages are designed for the analysis of brain imaging data sequences. There is a variety of software packages available for fMRI data analysis. Each has its advantages and disadvantages.

- BrainVoyager is a costly software package and it is a product of Brain Innovation B.V. (http://www.brainVoyager.com/index.html).
- AFNI (Analysis of Functional Neuroimages) is a free software package (http://afni.nimh.nih.gov/afni)
  (Heuttel et al., 2009).

BrainVoyager was used in the current fMRI study because it is fast and user friendly. Automatic brain segmentation, surface reconstruction, cortex inflation and flattening can also be done by using this software. BrainVoyager also supports advanced data analysis of fMRI brain images. Open Graphics Library, the most widely used cross-language for 2D and 3D graphics rendering, is implemented in BrainVoyager software for rendering 3D computer graphics (surface module) (James, Rajesh, Chandran & Kesavadas, 2014)

fMRI is a new, non-invasive technique, but also a crucial technique for understanding the functioning of the brain (Heuttel et al., 2009). Functional neuroimaging techniques are becoming important tools for rehabilitation research and can be used to determine the effects of brain injury or disease on the brain. Non-invasive fMRI can provide valuable information concerning the basic pathophysiology of HIV/AIDS in certain brain areas and of the potential neurotoxic actions of ART (Ances et al., 2010). For these reasons and proven benefits, fMRI was used in this study.

2.5.3. The use of fMRI for assessing the influence of HIV/AIDS on CANS

fMRI is often used in auditory research. It indicates metabolic changes in certain regions of the brain after auditory stimulation. Valuable screening tools such as fMRI can help with early detection of CAPD. The earlier the detection of CAPD, the quicker intervention can be instituted to reduce the impact on an individual (Chermak & Musiek, 2013). This non-invasive technique can also help to monitor the progress of an individual with CAPD.

The development of effective treatments for HIV/AIDS have improved worldwide survival rates but also increased the cognitive and functional impacts of the disease. The latter now represent a significant public health issue in South Africa. Future research efforts should focus on the efficacy of cognitive and behavioural remediation strategies of HIV/AIDS as well as standardisation of neuropsychological assessments that assist in measuring problematic cognitive symptoms (Vally, 2011). People with CAPD present less extensive activation in the brain areas that are associated with the attention areas in the brain (Pluta et al., 2011).

Neuroimaging techniques are beginning to capture the changes in brain activation in humans that accompany impaired or enhanced auditory experience. There is a role for imaging techniques to be used to detect abnormal brain activity, which can provide a more evidence based diagnosis if it is used together with the patient's history, detailed audiometry, electrophysiological testing, and neuropsychological testing (Moore, 2002; Micallef, 2015).

2.6. Conclusion

The literature review presented above outlined the main strengths and limitation of the research that has been conducted to date. It is clear from the literature review that South Africa is facing a significant challenge in addressing the HIV/AIDS pandemic that is affecting the population. The chapter also deals with the theoretical overview of the influence of HIV/AIDS on the structure and functioning of the auditory system and the typical related audiological manifestations. Finally, the chapter includes the principles of MRI and fMRI, as well as the value of the procedure in the functional assessment of the CANS. The next chapter (chapter three) contains a discussion of the methodology used for data collection, preparation and analysis, apparatus, subjects, the research design, and the procedures selected.

CHAPTER 3
METHODOLOGY

"It is important to get results from experiment but the most important is the process in getting that results."
(Dr Nik Ahmad Nizam)

3.1. Introduction

Research is always aimed at the solution to a problem. The process of research, according to Leedy (1993), in Fouché & Delport, (2011), is largely circular in configuration, in the sense that it begins with a problem and it ends with the problem solved. Rigorous researchers bear in mind, however, that the Russian proverb *trust, but verify* underpins science in that results should always be subject to challenge from experiment. Research is based on some underlying philosophical assumptions about what constitutes 'valid' research, and which research methods are appropriate for the development of knowledge in a study. In order to conduct and evaluate any research, it is important to know what these assumptions are. It is essential that the appropriate and relevant research methodology is selected and applied, since the methodology often determines the outcome of the study (Myers, Gleason, Yoon & Kung 1997).

In addition to a discussion of the main aim and sub-aims, this chapter contains a focused and systematic examination of the underlying philosophical assumptions as evidenced in the hypotheses, applicable research design, and the research process including participant selection (population, sample, and information), description of participants, pilot study, material and apparatus for participant selection, procedure for participant selection, and data collection. The procedure for data interpretation and analysis, as well as the analysis of data are also discussed in this chapter. Reporting of ethical approval (ethics clearance) and informed consent in clinical research articles involving human subjects are mandatory. This chapter ends with a conclusion.

3.2. Aims of the study

Main aim: To determine the response of the central auditory nervous system (CANS) to sound in normal hearing adults with and without HIV/AIDS, using fMRI.

- **Sub-aim one:** To determine the response of the CANS (ROI)[1] to nonsense syllables in normal hearing adults with and without HIV/AIDS, using fMRI.
- **Sub-aim two:** To determine the response of the CANS (ROI) to warble tones in normal hearing adults with and without HIV/AIDS, using fMRI.
- **Sub-aim three:** To compare the response of the whole brain to nonsense syllables and warble tones in participants with normal hearing with and without HIV/AIDS.

3.3. The hypotheses

A hypothesis is a tentative suggestion or preliminary statement about the relationship between two or more variables in the population being examined. The task of the researcher is to examine the research problem and find an appropriate explanation for it (De Vos, Strydom, Fouché & Delport, 2011). A quantitative study will start with a hypothesis, and then provide evidence to prove it true or false (Delport & de Vos, 2011). The **null hypothesis (H_0)** predicts that there is no relationship between two measured phenomena. The **alternative hypothesis (H_1)** is a supplement to the null hypothesis: it will predict a direction of change, an alteration, and a difference (Brewer & Stockton, 2010). The following hypotheses were proposed for this study:

- **$H1_0$:** There is **no difference** between the response (as measured with fMRI) of the CANS (ROI) to **nonsense syllables** in normal hearing adults with HIV/AIDS and in normal hearing adults without HIV/AIDS.
- **$H1_a$:** There is a **difference** between the response (as measured with fMRI) of the CANS (ROI) to **nonsense syllables** in normal hearing adults with HIV/AIDS and in normal hearing adults without HIV/AIDS.

[1]region of interest (ROI)

- **$H2_0$:** There is **no difference** in the response (as measured with fMRI) of the CANS (ROI) to **warble tones** in normal hearing adults with HIV/AIDS and in normal hearing adults without HIV/AIDS.
- **$H2_a$:** There is a **difference** in the response (as measured with fMRI) of the CANS (ROI) to **warble tones** in normal hearing adults with HIV/AIDS and in normal hearing adults without HIV/AIDS.
- **$H3_0$:** There is **no difference** in the response of the whole brain to nonsense syllables and warble tones in participants with and without HIV/AIDS.
- **$H3_a$:** There is a **difference** in the response of the whole brain to nonsense syllables and warble tones in participants with and without HIV/AIDS.

3.4. Research design

A research design is the outline of the overall research strategy that is to be used to ensure that the research problem is addressed effectively (Fouché & De Vos, 2011). The current research was **quantitative**, **applied,** and **exploratory** in nature (Leedy & Ormrod, 2010; Fouché & De Vos, 2011).

Quantitative methods emphasize objective measurements and the statistical, mathematical, or numerical analysis of data. Quantitative research focuses on gathering numerical data to explain a particular phenomenon (Babbie, 2013). Quantitative research implies an approach using variables. A sound quantitative design should only manipulate one variable at a time. The variable in this study was a two-valued (dichotomous) variable using people with HIV/AIDS and people without HIV/AIDS. Quantitative data can be statistically analysed to answer the research question. Thus, during this study, a quantitative approach was used primarily to analyse and interpret whether HIV/AIDS has an effect on the response of the CANS to sound when using fMRI (Fouché & De Vos, 2011). The advantage of quantitative research is that the methods used enable the comparison of measurements and data (Durrheim, 2002).

Applied research deals with solving practical problems, so as to provide evidence based practice (Fouché, 2011). The outcomes of this study may enhance diagnostic protocols, and indirectly contribute to improving service delivery to persons infected with HIV/AIDS.

Exploratory research techniques are used to investigate an area that has not yet been sufficiently investigated (Struwig & Stead, 2001). When conducting an exploratory research study, it is necessary to obtain a clear picture of a situation, phenomenon, community, or person. The need for such a study could arise from the need for more or basic information about the new phenomenon (Fouché & De Vos, 2011). This research design is typically used when studying something of new interest. The current study investigated a particular phenomenon in a population where it has not been researched before; in fact, limited published research has been conducted on HIV/AIDS in South Africa (Khoza-Shangase, 2010), especially using fMRI. In addition, this study aimed to examine the feasibility of the field of study, and to advance the technique or method to be used in this study (Fouché & De Vos, 2011).

This is a **comparative design** because two groups were used in this study: an exploratory group and a control group. The exploratory group had a specific condition namely HIV/AIDS, whereas the control group did not (Fouché & De Vos, 2011). Specific responses of the central auditory nervous system were tested, and the results were compared in each instance with data from the comparison group (Fouché & De Vos, 2011).

3.5. Ethical clearance and informed consent

As human participation was necessary in this study, ethical standards were observed in order to adhere to best practice in research. Participants were not influenced to participate in the study, and were fully informed about its purpose and procedure (Leedy & Ormond, 2013). Through research, information can be obtained, and new methods created for treating diseases and disorders. This can only be done in an honest, responsible, open, ethical, and justifiable manner (Hegde, 2003). The perceived social unacceptability of HIV/AIDS is a sensitive and delicate issue to be discussed, examined, or dealt with, therefore participants were especially vulnerable. Although some research offers the prospect of benefitting the research participants directly, some research (as in this case) does not (Wolf & Lo, 2001). Selecting the research population for this study involved a number of steps and criteria to identify the sample of people who were willing to participate in this research. Throughout the selection process, participants' files were used to determine their HIV/AIDS status. During this process confidentiality was maintained at all times (Blanche & Durrheim, 2006).

Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Humanities, University of Pretoria, and from the South African Military Health Services Research Ethics Committee (See appendix E).

The following **ethical considerations** were upheld in the study:

- ***Autonomy***

The Oxford Advanced Learner's Dictionary (Hornby & Deuter, 2015) defines the term 'autonomy' as "the participant's own perception of his or her own affairs". In research, 'autonomy' means strictly voluntary participation (Leedy & Ormond, 2013). The components of autonomy include aspects such as the participants' right to withdraw, privacy, confidentiality and anonymity, disclosure of information, debriefing of participants, and ethical clearance (informed consent) (Babbie, 2013). These aspects (as they relate to the current study) are described in Table 9.

Table 9: Components of autonomy as applied in this study

Component	Description
Informed consent	The participants were fully informed by means of a letter of informed consent (Appendix B) about all the risks and benefits involved (Strydom, 2011).
Withdrawal of participants	Participation was voluntary. The participants could withdraw at any time from the study (Babbie, 2013). This was explained in the letter of informed consent (see Appendix B).
Privacy, confidentiality and anonymity	Participants' privacy was respected. All documents and information were treated as confidential (Strydom, 2011). The information obtained from this study was treated as highly confidential. Names of participants were not used in this thesis or in articles or presentations compiled after the completion of the study. A specific code was allocated to each participant, and these were used during the data collection and data analysis.
Disclosure of information	Participants were informed by means of a letter of consent that the information obtained from the research study would be used for research purposes (Strydom, 2011). Personal details were not revealed.
Availability of results	The research findings will be available on request. Debriefing of the participants is a way of minimising harm (Strydom, 2011). In addition, a summary of results will be available to the participants on request, and the data gathered will be archived for 15 years.

- ***Beneficence***

The findings of the research study should benefit the participants as well as the general population (Hicks, 2004). The interests and well-being of the participants were respected, and they were not exposed to any harm. The components of beneficence (French *et al.*, 2001) are described in Table 10.

Table 10: Components of beneficence as applied in this study

Component	Description
Competency	The researcher participating in the study is registered with the Health Professions Council of South Africa and is licensed to perform the various examinations and procedures. The hearing testing was performed by audiologists with necessary qualifications and registrations.
Relevance	This study is relevant and valuable for future technology and research. Obtaining this information will help to appropriately diagnose hearing problems caused by central auditory involvement, and to ensure that these participants receive suitable rehabilitation for their hearing disorders.
Risks	The risk incurred by participating in this study did not exceed the risk of normal daily living. Routine clinical procedures were performed.
Discrimination	Participants were not discriminated against on account of their age, gender, race, or economic status.

- ***Justice***

On principle it is imperative that all participants be treated uniformly and honestly (Hicks, 2004). The researcher did not amend or disguise the actual findings of the research project (Strydom, 2011).

- ***Infection control***

A participant infected with HIV/AIDS is already compromised. Infections associated with HIV/AIDS, such as bacterial pneumonia, tuberculosis and respiratory tract infections (otitis media, otorrhea, sinusitis, or tonsillitis) (Hoffmann, Rockstroh & Kamps, 2007) can easily spread to other participants. It was very important, therefore, that arrangements had to be in place to protect all participants without as well as participants with HIV/AIDS (Kemp &

Bankaitis, 2000). A new pair of surgical gloves was used when dealing with each participant, and the equipment was sterilized after each assessment.

3.6. Participants: Population and sample

The participants in the exploratory group were recruited from a government hospital's adult HIV/AIDS clinic. The participants who attended this clinic had already been diagnosed with HIV/AIDS, and were seen for general medical management. A number of these participants were receiving ART treatment and were monitored at the clinic.

A control group serves as a comparison group that comes from the same population but that does not have the condition that defines the exploratory group of people. The condition might be a disease – in this case, HIV/AIDS (Bowers, House & Owens, 2013). Data collected during the control phase was compared with the data collected in the exploratory phase (Babbie, 2013). The participants in the control group were recruited from the tertiary referral hospital as well as a tertiary institution and acted as a reference for comparison. The sample groups of participants therefore consisted of two groups: the exploratory or experimental group (HIV/AIDS-positive participants) and the control group (HIV/AIDS-negative participants). A randomised sample of participants attending the clinic was used to allow each participant an equal chance of participating (Blanche et al., 2006). In Appendix G statistical tests will be done to control for the normal distribution of groups, racial distributions and age disparity.

3.6.1. Participant selection criteria

The participant selection criteria were an important part of the investigation as it ensured the inclusion of suitable candidates in the study, which enhanced the reliability. The data were collected from a selection of individuals who belonged to a large group of people infected with HIV/AIDS. To enhance validity and reliability the results obtained from this group were compared to a control group without HIV/AIDS. Table 11 summarises the general participant inclusion criteria to which participants with HIV/AIDS had to adhere to be included in the study (Appendix C, Section F).

Table 11: General selection criteria for the participants

Criterion	Description	Justification
Informed verbal consent	Participants had to give informed consent. (See Appendix B)	Each participant who participated had to give full voluntary consent before the study commenced. According to the International Guidelines on HIV/AIDS and Human Rights, informed consent is an ethical principle in the medical profession (Heywood, Macaskill & Williams, 2010).
Age	Participants had to be between 18 years and 50 years.	These age groups were selected to ensure that participants were old enough to provide informed consent, and also to ensure that participants with a hearing disorder associated were degenerative changes related to ageing were not included. Age-related deafness is most often from the age of 50 years and older (Williamson, 2004).
Language	Participants had to be able to communicate in Afrikaans and/or English.	This ensured clear communication during consent, and instructions during test procedures.
Geographical area	Participants who attended the Adult HIV/AIDS clinic in Pretoria were approached to be part of the study.	This facility has a large population with HIV/AIDS. This facility was available and easily accessible to the researcher.
Middle ear function	Participants had to have normal middle ear function as determined by otoscopy and tympanometry: Type A tympanogram (Stach, 2010:265)	Abnormal middle ear functioning could negatively influence hearing status and results (Stach, 2010).
Hearing status	Participants had to have normal results for: • Pure tone air conduction test 0-25 dB (Stach, 2010; Khoza & Ross, 2002). • No family history of a hearing disorder	A hearing loss is an additional variable that can influence the fMRI findings (Stach, 2010).
Neurological status	The neurological status was ascertained by a questionnaire (See Appendix C).	Participants with a previous or ongoing history of neurological illness were excluded from the study, as this might affect auditory fMRI findings.
MRI suitability	Participants were not included if they had any of the following: • vascular aneurysm clips • heart valves • metallic foreign bodies • pacemakers • cochlear implants • people with claustrophobia This was determined by means of a questionnaire (See Appendix C).	The MRI environment can be hazardous to participants if they indicated any of the listed conditions (Heuttel et al., 2009).
Specific disorders that may influence auditory fMRI outcomes	Participants should not have presented with the following, nor had a once-off incidence of the following: • Meningitis • Encephalitis • convulsions/epilepsy • facial nerve palsy / cerebral palsy • dysphasia • voice problems (neurogenic) • speech problems (neurogenic) • stroke • head trauma • multiple sclerosis • psychiatric disorders • drug and substance abuse • coma	These variables can influence the outcomes of the auditory fMRI, thereby making it difficult to distinguish between the possible effects of HIV/AIDS on the CANS (Stach, 2010). (See Case Report Form in Appendix C).

The specific participant selection criteria for the **experimental** and the **control groups** were as follows (Table 12):

Table 12: Participant selection criteria for the experimental group and control group

Criterion	Description	Justification
HIV/AIDS status	Participants must have been diagnosed with HIV/AIDS, based on the results of a blood test (standard antibody HIV/AIDS – enzyme-linked immunosorbent assay (ELISA) HIV serology test must have been less than 3 months old	Participants with HIV/AIDS were included in order to determine whether the central auditory system nervous system (CANS) was affected by the disease. The blood test results identified the HIV/AIDS status and classification of each participant. All categories (A, B, C) of the CDC (Centre for Disease Control in the USA) conditions associated with HIV/AIDS infection were included in the sample, and grouped ad hoc in specific classifications (Hoffmann, et al., 2007).
Experimental group	serology need to be positive for HIV/AIDS	
Control group	serology need to be negative for HIV/AIDS	

- **Criteria for the selection of participants in the experimental group**

The participants in the experimental group were required to comply with the general selection criteria (Table 12), and in addition these participants had to be HIV/AIDS-positive (Table 12).

- **Criteria for selection of participants in the control group**

The general selection criteria set out in Table 12 were applied during the selection of suitable participants for the control group in this study. In addition, these participants had to be HIV/AIDS-negative (Table 12).

3.6.2. Material and apparatus for the selection of participants

Participant selection material was used to obtain all the necessary information from the participants. This was done in a structured manner in order to provide the researcher with the means and motivation to exclude or include a participant, and to collect the necessary information for statistical analysis. The data selection material consisted of a **letter of informed consent** (Appendix B), an **information leaflet** about fMRI scans (Appendix A), and

a **case report form** (Appendix C) that was completed by the principal investigator and a co-investigator. The case report form was in the format of a questionnaire and data recording form with six sections:

Section A

This section aimed at obtaining **biographical information** from each participant: age, gender, race, and education. This information was regarded as essential for an adequate description of the research sample. The participants were asked to specify their home language in order to determine how best to communicate with them. It was necessary to obtain the body weight of each participant, as this was required for radio-frequency (RF) safety purposes for the MRI scan. The most important effect of RF exposure is the heating of tissue, and so the correct weight had to be entered to prevent over-heating from occurring (McRobbie, et al., 2017).

Section B

In this section, the questions ascertained each participant's **medical history**, medical conditions, and HIV/AIDS status. Participants also had to indicate whether they were under ART or TB treatment, and also the duration of such treatment up to the time of testing.

Section C

The questions in this section about **MRI compatibility** were adapted from the MR Questionnaire of the American College of Radiology White Paper on MR safety (Kanal, Borgstede, Barkovich, Bell, Bradley, Felmlee, Froelich, Kaminski, Keeler, Lester & Scoumis, 2002). The information obtained in this section was used to determine the suitability of performing MRI on the participant. Due to the fact that the MRI environment can be hazardous, the screening process included questions about previous surgery, prior exposure to metallic slivers or fragments that might have entered the eye, and pregnancy. The questionnaire also determined whether the participant had an implant in his/her body, foreign material, or any device or other items that are considered to be hazardous in the MRI environment, or that might make the achieving of high image quality difficult (Heuttel et al., 2009). This includes any device that is electrically, magnetically, or mechanically activated.

The screening questionnaire was also used to obtain additional information on a variety of topics related to the safe performance of the MRI procedure. For example, questions included previous adverse reactions to contrast media, which could alert the researcher to potential problems (Sawyer-Glover & Shellock, 2000).

Section D

This section was developed to **examine and record clinical information regarding the auditory system**. These questions included the participant's history of medical conditions that might have caused hearing loss, or that could have had an influence on the audiometric test results. The questions assisted sample selection by determining whether participants adhered to the selection criteria. It was important to establish whether the participant had any previous illnesses or medical conditions. This was to eliminate any other possible causes of hearing loss unrelated to HIV/AIDS. The literature indicates that numerous other conditions can lead to hearing loss (Matas et al., 2014).

Section E

This section included the Audiology examination that included the **hearing test results** that were obtained to determine the hearing ability of the participant. The following audiometric tests were performed, as they were considered essential (according to Stach, 2010): an otoscopic examination, acoustic immittance testing, and pure tone air and bone conduction testing. This section was important as only participants with normal audiometric results were included in this study. In order to confirm normal hearing thresholds in each participant, conventional pure tone audiometry (250 Hz – 8000 Hz air conduction) was performed on all participants, mostly had normal hearing thresholds but the participants with abnormal hearing results were referred for further diagnostic testing at the ENT department. The apparatus used for the hearing tests are indicated in Table 13 (Stach, 2010).

Table 13: Apparatus for hearing assessment as part of participant selection

APPARATUS	SPECIFICATION
Audiometric apparatus	
Heine mini 2000 CE Otoscope	For optimum performance, the otoscope was used with sterilised speculums.
GSI tympstar immittance meter *Calibration date: January 2012* *Calibration date: January 2013*	The immittance meter was calibrated prior to testing according to the requirements of South African National Standards (SANS) SANS 10154-2000 to ensure that the results were reliable and accurate. All the probes were sterilised before use, and suitable probes were selected for each participant to ensure a proper fit.
GSI 61 two channel clinical audiometer *Calibration date: January 2012* *Calibration date: January 2013*	The audiometer was calibrated prior to testing according to the requirements of SANS 10154-2000, so that correct and reliable results were obtained. The assessments were done in a double-walled soundproof test room.
Soundproof booth	The room was soundproof, according to SANS 10082:2006 standards.
TDH-39 earphones	Sterilisation of the earphones was done before each examination.
Sterilisation equipment	
Milton sterilisation fluid	The researcher ensured that the expiry date was valid.
Webcol alcohol swaps	The researcher ensured that the expiry date was valid.

Section F

This section included all the results obtained and then the final evaluation was done to determine if the participant was eligible for the study. For this study participants were considered to have normal hearing when they could hear pure tones of 25 dB or lower across all frequencies (Martin & Clark, 2015; Khoza & Ross, 2002).

3.6.3. Participant selection procedure

A poster (Appendix D) with details about the study was placed at the hospital. The size of the participant group depended on the number of appropriate participants who consented to take part in the study. The diagram in Figure 10 indicates the *procedures* for participant selection and data collection for this research study, in the order in which they occurred, and it classifies all the *stages* of the research process in the participant selection and data collection of this research project. It is important to realise that the specific selection and pre-screening of participants were necessary to be able to select participants who adhered to the selection criteria (inclusion criteria) for the fMRI study.

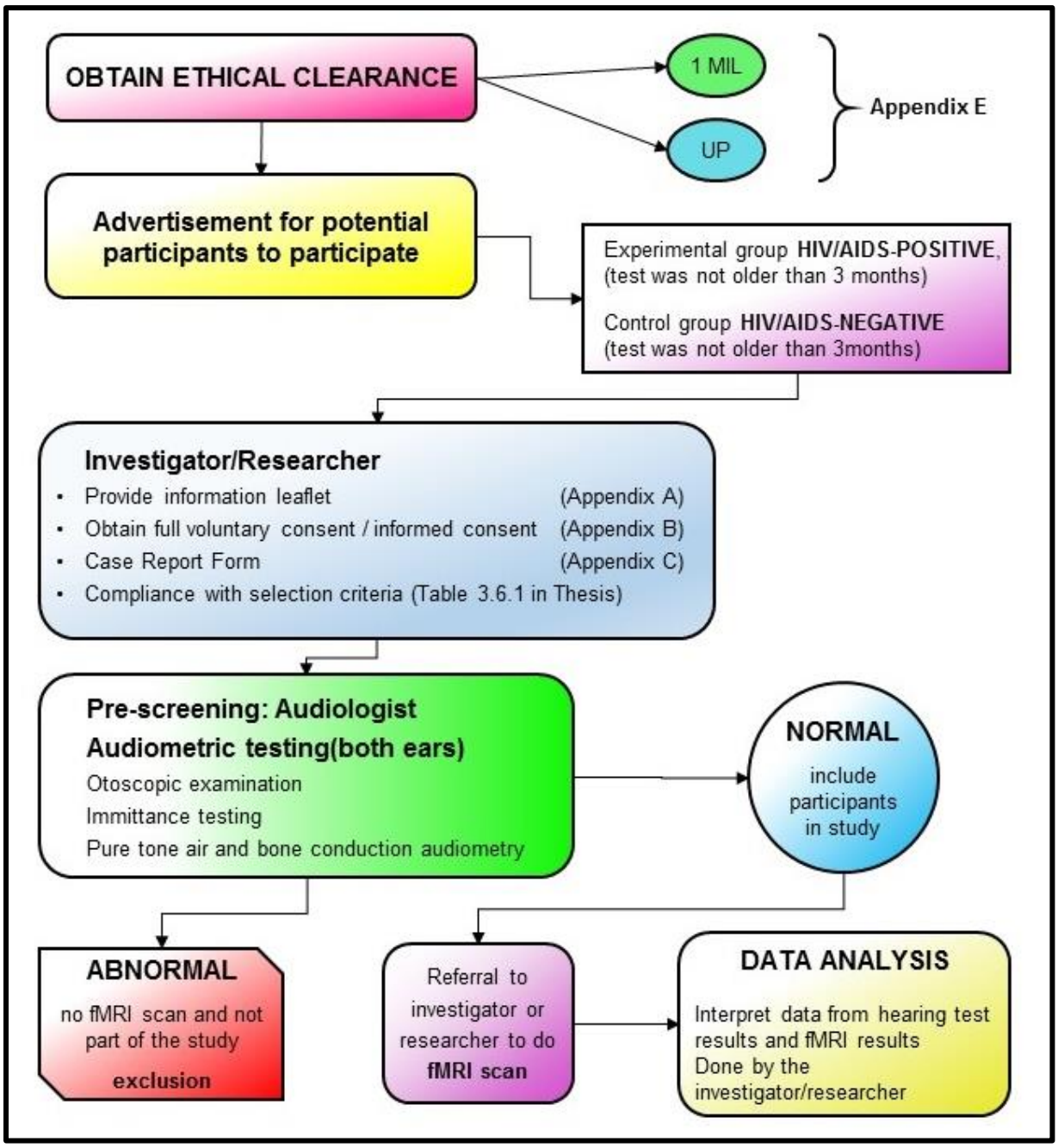


Figure 10: Diagram of the different procedures and stages of participant selection and data collection

As indicated in Figure 10 all participants who met the selection criteria were required to attend the ENT (Ear, Nose and Throat) Department at a hospital in Pretoria. The HIV/AIDS group attended the Infectious Disease Clinic at the hospital in Pretoria. All the participants were tested for HIV/AIDS to confirm their HIV/AIDS status. They were requested to participate in the study on a voluntary basis, and – if they agreed – they were asked to sign a consent form in order to participate (Appendix B). The content of a **letter of informed consent**

(Appendix B) was also explained verbally to each participant, who was informed of his/her role and the importance of the information. An assurance was given that all information and results would be kept confidential, and that participation in the research would be valuable, appreciated, and voluntary. The participant was then asked to sign the letter, thus agreeing to participate. Pure tone audiometry as well as tympanometry was performed on all the participants to ensure that participants had normal audiometric results.

In the case report form (Appendix C), the sample characteristics and immunological information were requested from the participants. The results of each HIV/AIDS and control group participant's immunological test had to be not older than three months. The participants in the HIV/AIDS group were a randomised sample of participants attending the clinic at the tertiary referral hospital. This allowed each participant an equal chance of participating. The participants in the control group were recruited from the tertiary referral hospital as well as a tertiary institution and acted as a reference for comparison. The control participants underwent an HIV/AIDS screening test (rapid test device), and participants with negative results were enrolled in the study. This rapid test device has been authorised by the FDA and WHO. It is accurate, with a 99 per cent sensitivity and specificity, and the results are instantly available (Bekker, 2010). The sample groups of participants therefore consisted of two groups: the exploratory or experimental group (HIV/AIDS-positive participants) and the control group (HIV/AIDS-negative participants) (Blanche et al., 2006).

3.6.4. Description of participants

A total sample of 27 participants (HIV/AIDS and control) comprised the study. The sample characteristics for the 27 recruited participants are summarised in Table 14 and Table 15. The independent sample test, t-test for equality of means in SPSS statistics, was used to compare the mean, median, and standard deviation for the age, gender, and years of education of the two groups. After the detailed description of the participants, the mean hearing thresholds of the participants will be described.

Description of participants

Table 14 contains the detailed description of the HIV/AIDS group and the control group in terms of gender, age (years), and education (years). A comparison of the means and standard deviations for the two groups reveals that an approximate match was obtained.

Table 14: Demographic characteristics of the two groups

Demographic Characteristics	Group	number of participants (N)	Minimum male/female	Maximum male/female	Mean	Std. Deviation	p value
Gender	HIV/AIDS	12 (4 male 33.3%)	1 male	4 male	1.67	0.49	
	Control	15 (5 male 33.3%)	1 male 1 female	5 male 10 female	1.67	0.49	
	Group Total	**27 (male/female)**	**1 male 1 female**		**1.56**	**0.506**	**p=1.000**
Age (years)	HIV/AIDS	12	23	43	32.42	6.54	
	Control	15	21	34	24.93	4.43	
	Group Total	**27**	**21**	**43**	**28.26**	**6.56**	**p=0.003***
Education (years)	HIV/AIDS	12	11	15	12.33	1.07	
	Control	15	10	16	14.40	1.55	
	Group Total	**27**	**10**	**16**	**13.48**	**1.7**	**p=0.001***

*Note. Significant difference at the $p \leq 0.05$ level

The distribution of male and female was exactly matched. Both the HIV/AIDS group and the control group had 33.3 per cent male and 66.7 per cent female participants respectively, as shown in Table 14 and Figure 11. There were no significant differences (p = 1) found between the two groups.

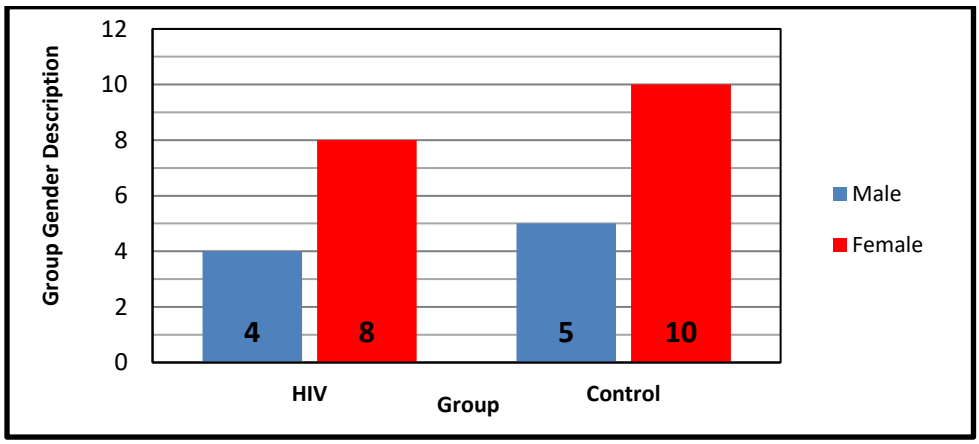


Figure 11: Group gender description of the two groups

The **age** (in years) of the 12 HIV/AIDS participants (mean ± standard deviation) was M ± SD = 32.42 ± 6.54 years, and of the fifteen control participants it was M ± SD = 24.93 ± 4.43 years. There was a significant difference (p = 0.003) between the two groups. This did not have an influence on the results, because all the participants still meet with the specified selection criteria.

The **education** (in years) of the 12 HIV/AIDS participants was M ± SD = 12.33 ± 1.07 years, and of the 15 control participants M ± SD = 14.40 ± 1.55 years. The control group had more years of education than the HIV/AIDS group. There was a significant difference (p = 0.001) between the two groups. This did not influence the results, as the participants still met with the selection criteria.

The immunological information for the 12 **HIV/AIDS** participants was summarised in **Table 15** and it also shows how closely the **HIV/AIDS naïve group** and the **HIV/AIDS group on ART** could be matched in terms of **gender**, **age (years)**, **education (years), ART (years)** and **CD4 count**.

Table 15: Demographic and HIV/AIDS related characteristics

Demographic and HIV/AIDS related characteristics	Group HIV/AIDS	Number of participants / years / count	Mean	Std. Deviation	p value
Gender	HIV/AIDS naïve	4 (1 male / 25.0%)	1.75	0.50	
	HIV/AIDS on ART	8 (3 male / 37.5%)	1.63	0.52	
	Group HIV/AIDS Total	12 (4 male / 8 female)	1.67	0.49	p = 1.000
Age (years)	HIV/AIDS naïve	4	29.25	7.14	
	HIV/AIDS on ART	8	34.00	6.07	
	Group HIV/AIDS Total	12	32.42	6.54	p = 0.233
Education (years)	HIV/AIDS naïve	4	11.75	0.50	
	HIV/AIDS on ART	8	12.63	1.18	
	Group HIV/AIDS Total	12	12.33	1.07	p = 0.119
ART (years)	HIV/AIDS naïve	4 (33.3%)	0	0	
	HIV/AIDS on ART	8 (66.7%)	2	1.31	
	Group HIV/AIDS Total	12	1.33	1.44	p = 0.007*

CDC categories HIV/AIDS group	Count	Number of participants	
Category 1	> 500	6	50.0%
Category 2	200 – 500	4	33.3%
Category 3	< 200	2	16.7%

* Note. Significant difference at the p ≤ 0.05 level

The distribution of male (HIV/AIDS naïve group and the HIV/AIDS group on ART) and female (HIV/AIDS naïve group and the HIV/AIDS group on ART) differed slightly. The HIV/AIDS naïve group had 25 per cent male and 75 per cent female participants and the HIV/AIDS group on ART had 37.5 per cent male and 62.5 per cent female participants respectively. There was no significant difference ($p = 1$) between the two groups. A comparison of the mean and standard deviation for the two groups reveals that an approximate match was obtained for age. HIV/AIDS naïve group $M \pm SD = 29.25 \pm 7.14$ and HIV/AIDS group on ART $M \pm SD = 34.00 \pm 6.07$. There was no significant difference ($p = 0.223$) between the two groups. The education (years) of the HIV/AIDS naïve group ($n = 4$) was $M \pm SD = 11.75 \pm 0.50$ years, and of the HIV/AIDS group on ART ($n = 8$) it was $M \pm SD = 12.63 \pm 1.18$ years. The HIV/AIDS group on ART had slightly more years of education than the HIV/AIDS naïve group. There was no significant difference ($p = 0.119$) between the two groups. A comparison of the mean and standard deviation for the two groups reveals that, as was to be expected, no match was obtained for years on ART (HIV/AIDS naïve group $M \pm SD = 0 \pm 0$, HIV/AIDS group on ART $M \pm SD = 2 \pm 1.13$). The difference between the two groups was significant ($p = 0.007$). The HIV/AIDS group as a whole was also divided in terms of CDC categories. In terms of CDC categories, the distribution of the HIV/AIDS group in CDC category 1 was 50% (n=6), CDC category 2 was 33.3% ($n = 4$) and the CDC Category 3 16.7% ($n = 2$).

Mean hearing thresholds

In order to confirm normal hearing thresholds in each participant, conventional pure tone audiometry (at the frequencies of: 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, 6000 Hz and 8000 Hz) was performed on all participants. In Section E of the Case Report form (Attachment C) the **hearing test results** that were obtained to determine the hearing ability of the participant was captured on the form and the Kruskal-Wallis test (one-way ANOVA on ranks) was a rank-based nonparametric test that was used in SPSS statistics (Heck, Thomas & Tabata, 2010). The mean hearing thresholds for the two groups (HIV/AIDS and control) using pure tone audiometry in both ears, are shown in Figure 12.

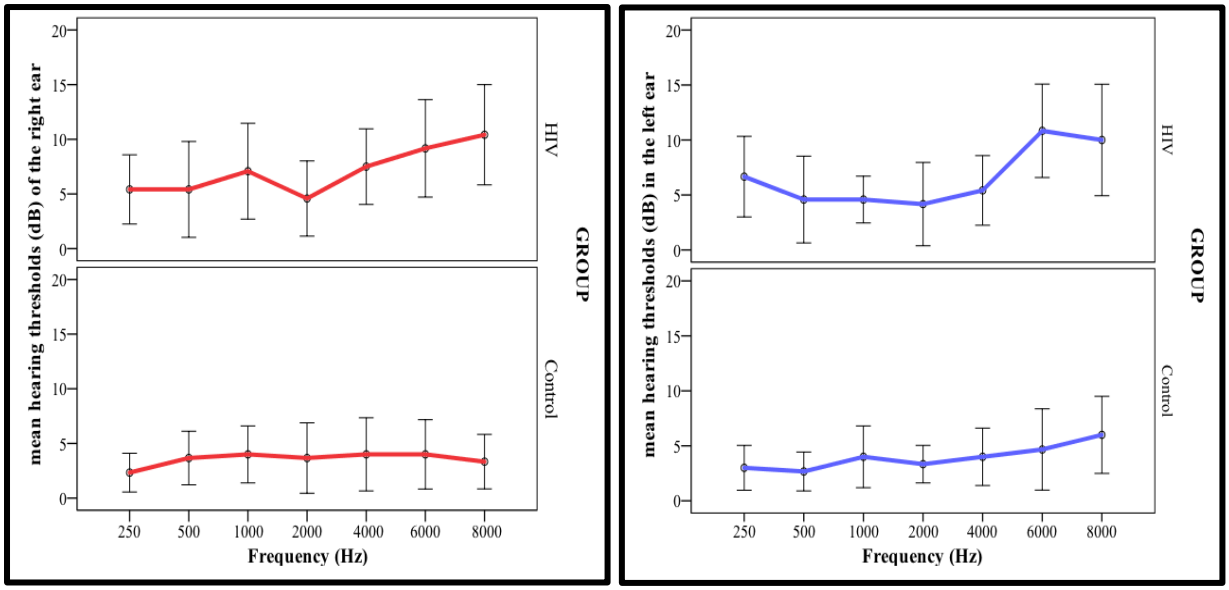


Figure 12: Mean hearing thresholds of the right (red) and left (blue) ear in both groups

The results were classified as 'normal' when the hearing thresholds were lower than, or equal to 25 dB (Martin & Clark, 2015; Khoza & Ross, 2002). The mean hearing thresholds are displayed per group (HIV/AIDS group and the control group) for both ears separately namely right (red) and left (blue). Although some variability in the hearing thresholds, all the participants results are within the required normal threshold.

3.7. Data collection

In this section the procedures used to collect the data for this study will be discussed. Firstly the **material and apparatus for data collection** (pilot study and this experimental study) see **Table 16** will be discussed and then the procedure, material and apparatus and results of the pilot study will also be discussed thereafter.

3.7.1. Material and apparatus for fMRI data collection

The material and apparatus described in Table 16 was used during the **pilot study** and **this experimental study** to obtain the data required.

Table 16: Material and apparatus for fMRI data collection

APPARATUS	JUSTIFICATION	SPECIFICATION
Magnetom Siemens Espree 1.5 T	This apparatus was used for functional magnetic resonance imaging. This is a neuroimaging technique that measures the physiological changes associated with neural activity in the brain. It is used with an MRI scanner to study brain function over a specific period (Huettel et al., 2009). This procedure helped to determine central auditory neural processing.	Since the MRI environment can be hazardous, each participant completed the necessary safety questionnaire. An MRI questionnaire (see Appendix E) was used with each participant to assess their suitability and safety before they entered the room to be scanned (McRobbie et al., 2017).
Magnet 1.5 T	The magnetic field is essential to MRI, providing the 'magnetic' in 'magnetic resonance imaging'. Strong static magnetic fields are aligned in certain nuclei in the human body in order to map the tissues of the human body (Huettel et al., 2009).	This was to ensure that the machine was in working order, and that quality assurance (QA) had been carried out on the equipment.
Quality Assurance (QA)	MRI QA is important to monitor and ensure the image quality. A phantom object was used. (McRobbie et al., 2017).	fMRI required additional QA based on the stability of the MR signal, SNR, and artifact level in EPI sequences (McRobbie et al., 2017).
Headphones for stimuli and two-way intercom	The headphones were used to communicate with the participant and to send auditory stimuli from a digital music player with a sound pressure level of 95dB which were presented to both ears simultaneously. Headphones were insulated to help reduce scanner noise (Bernal & Altman, 2001).	The headphones were cleaned after each participant had used them.
Radiofrequency coils	The coil has spatial proximity to the brain and provides high imaging sensitivity by generating and receiving energy (Huettel et al., 2009).	The head coil was placed on the surface of the head, adjacent to the surface of the scalp, for functional imaging (Huettel et al., 2009).
Computer hardware	The host computer displayed the image and processed it.	Statistical software was necessary for image processing and statistical analyses.
Computer software BrainVoyager QX post-processing software	fMRI detects the small changes in blood flow after an activity or task, allowing for BrainVoyager to analyse specific areas in the brain.	BrainVoyager QX is a software package that analyses and visualises the structural and functional MRI data (Goebel, 2012). (http://BrainVoyager.com)

3.7.2. Procedures of pilot study

A pilot study was conducted to ensure that the research material, research apparatus, and research procedures used in the study were appropriate, reliable, and valid. The pilot study was also conducted to identify and correct all possible deficiencies before starting the primary research. It therefore assisted the researcher with valuable guidelines for the planning of the

research. *"*The pilot study is indeed a prerequisite for the successful execution and completion of a research project*"* (Strydom, 2011:236).

The aims of the **pilot study** were:

- To assess the questions, procedures, and analysis procedure for data to ensure the reliability and feasibility of the study (Strydom, 2011);
- To identify errors in the procedures and instructions, and to recognise possible timing constraints;
- To identify vague or unclear information in the case report form, information leaflet, and the informed consent letter; and
- To observe the participants' reactions and behaviour during the data collection process, as this would help to identify any adverse responses (Welman, Kruger, Mitchell & Huysamen, 2005).

Seven participants with normal hearing were requested to perform a hearing task in response to auditory stimuli for an auditory functional MRI scan. The pilot study required that the case report form (see Appendix C) be completed and the letter of informed consent (see Appendix B) to be read through and signed. The participants were expected to provide feedback and to evaluate any positive or negative aspects of the procedures followed in the pilot study. This was used to determine the study's feasibility, and to guide the researcher in correcting or refining the questions for the purposes of clarity and understanding (Andrew, Davis & Kraemer, 2011)

Material and apparatus used for fMRI data collection for the pilot study

The apparatus described in **Table 17** was used during the pilot study to obtain the data required to proceed with the study.

Results of the pilot study

The results of the fMRI pilot study indicated satisfactory outcomes for all the participants. The participants stated that the instructions were clear to follow. Strategies for data processing were tested and evaluated. The data collection process for the audiometric testing took 45 minutes, and the fMRI scan took 45 minutes. The protocol used for the pilot study was therefore satisfactory and no changes were made to the protocol during the data collection.

3.8. Procedure for data collection for the experimental study

In Figure 13 the experimental block fMRI task design, data acquisition, MR protocol, pre-processing of fMRI data and preparing fMRI data for statistical analysis.

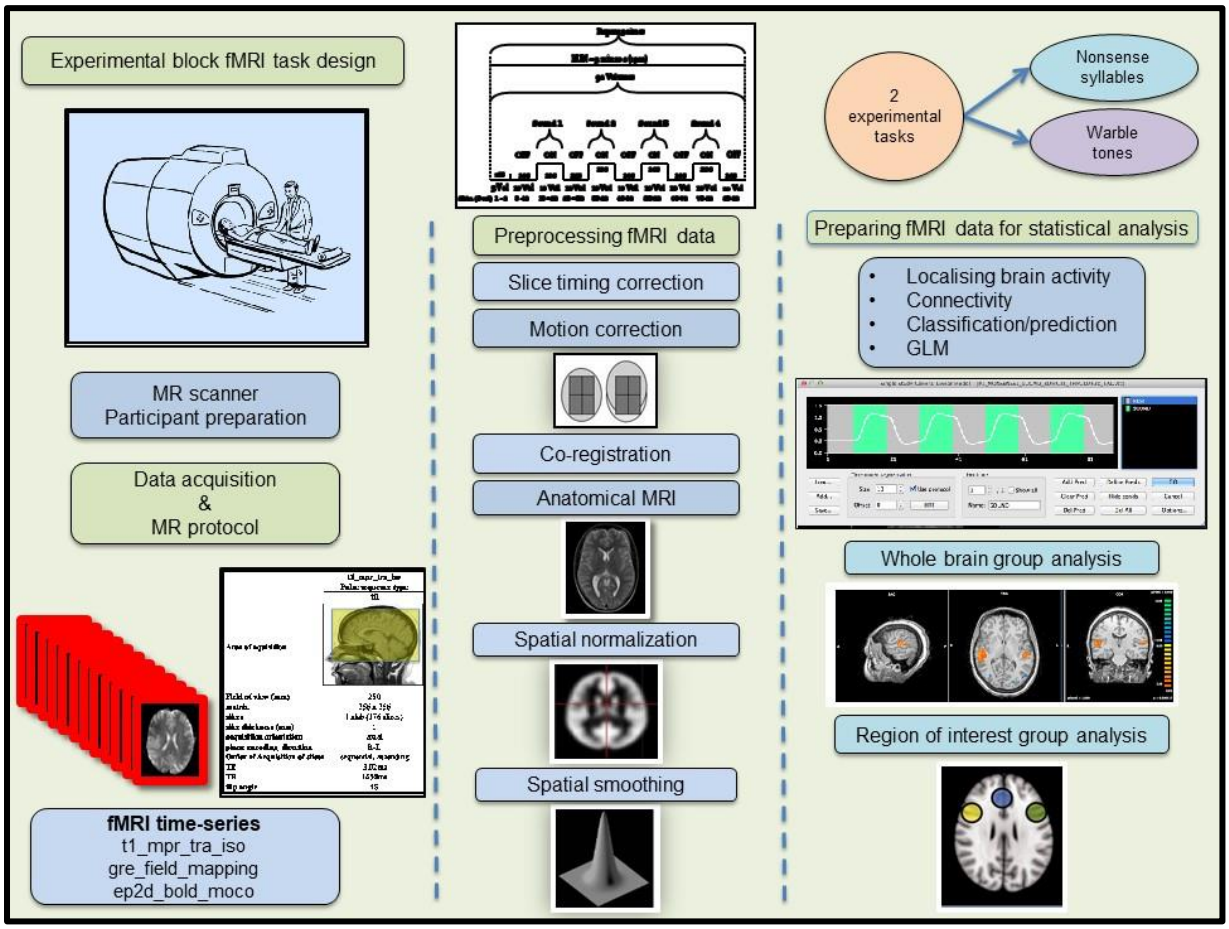


Figure 13: Key stages in fMRI data collection, processing, and data analysis

The key stages will be discussed in more detail below:

3.8.1. Functional MRI experimental tasks

Structural and fMRI images were acquired using a 1.5T Siemens Magnetom Espree. Earphones were fitted on the participant's head to cover both ears for the auditory stimuli that were presented to both ears. During the fMRI scan, participants were instructed to lie with their eyes closed and listen to two different listening tasks binaurally, the auditory stimuli with a sound pressure level of 95 dB were presented from a digital music player outside the scanner room but the cables were connected to the acoustic generator and the plastic tubes connected to the Siemens headphones. Twenty seconds blocks of stimuli were alternated with twenty seconds blocks of silence (see Table 17 & Figure 14) (Suzuki et al., 2002).

Participants were instructed to perform the following two different listening (stimuli) tasks:

- **Nonsense syllables task** (auditory consonant-vowel phoneme combinations such as ta, da, ka, ba, pa, and ga) (Scott & Feroze, 2010). Non-words were obtained from a list of six nonsense syllables (Table 120) prepared by Richard H. Wilson, PhD, Senior Research Career Scientist Audiology, Medical Center Mountain Home, Tennessee, in 2010 and available on a CD-ROM. During each stimulus block, syllables from the list were presented in random order for 20 seconds at a rate of one syllable per second. The series of syllables presented during each stimulus block in the present study are listed in Table 170 (Suzuki et al., 2002).

Table 17: Monosyllables presented during the nonsense syllables acquisition

6 nonsense syllables in dichotic format, one syllable per second					
1	TA	4	BA		
2	DA	5	PA		
3	KA	6	GA		
Sound 1	PA KA GA KA GA PA PA BA PA TA BA PA PA TA GA TA BA BA GA TA			20	seconds
Sound 2	PA KA KA BA KA TA BA PA BA TA BA KA BA PA DA DA KA PA TA KA			20	seconds
Sound 3	BA TA TA KA PA GA DA DA TA PA DA BA GA GA PA DA GA BA TA BA			20	seconds
Sound 4	BA GA BA GA DA KA TA KA TA TA PA DA PA KA DA BA BA TA GA PA			20	seconds

As shown in Table 17, the nonsense syllables were randomly presented to the participants at a rate of one per second. The nonsense syllable lists consisted of six syllables: *ta, da, ka, ba, pa* and *ga*. Earphones were fitted on the participant's head to cover both ears for the auditory stimuli that were presented to both ears. The manner of presentation enabled passive listening (hearing the sound without engaging with speaker) with both ears (Suzuki et al., 2002). During the fMRI scan, participants were instructed to lie with their eyes closed and listen to the binaural auditory stimuli comprising nonsense syllables.

In Figure 14, the number of experimental sessions and volumes[2] acquired per session are presented. During the fMRI scan, participants were instructed to lie with their eyes closed and listen to the binaural auditory stimuli comprising 'nonsense' syllables. Twenty seconds blocks of nonsense syllables were presented, alternated with 20 seconds blocks of silence (see Figure 14).

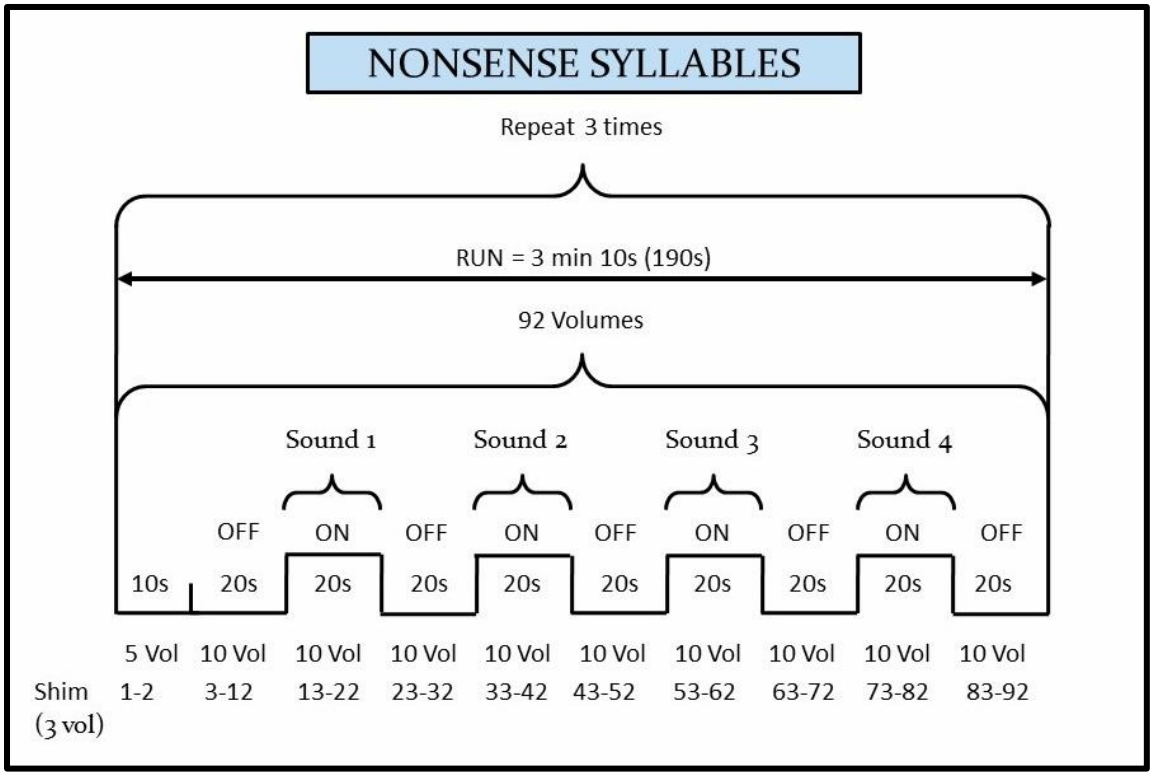


Figure 14: Nonsense syllables block design

[2]The use of the word 'volume' in this fMRI study indicates that, in a typical fMRI session, a 'low resolution functions volume' of the entire brain is acquired every few seconds (Heuttel *et al.*, 2009).

Each fMRI scan series lasted for three minutes and 10 seconds, and three scan series were collected from each participant. Within each scan series, single nonsense syllables were presented at one second per syllable (total 20 syllables / 20 seconds) trials using a block design (Suzuki et al., 2002).

Warble tones refer to frequency-modulated tones where the frequency varies periodically several times per second over a small range. The warble tones were randomly presented to the participant. The following five frequencies: 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, and 8000 Hz at ten seconds per frequency. In the present study an alternating high and low frequency tone was presented for 10 seconds each during each stimulus block of 20 seconds with the difference in frequency between the two tones varying between blocks (Valente (2002); Joanisse & DeSouza (2014)). The manner of presentation enabled passive listening with both ears (Bernal & Altman, 2001).

The two conditions, silence (baseline) and warble tones, were interleaved, duration 20 seconds per block, with each block repeated four times. The warble tones used in the present study are listed in Table 18.

Table 18: fMRI experimental task 2

Warble tone		**Sound 1**	8 000 Hz	10 seconds
			1 000 Hz	10 seconds
5 tracks: frequency range		**Sound 2**	1 000 Hz	10 seconds
1	500 Hz		500 Hz	10 seconds
2	1 000 Hz	**Sound 3**	8 000 Hz	10 seconds
3	2 000 Hz		2 000 Hz	10 seconds
4	4 000 Hz	**Sound 4**	4 000 Hz	10 seconds
5	8 000 Hz		500 Hz	10 seconds

The warble tones were prepared by Richard H. Wilson, and were available as tracks on a CD-ROM. The tones were presented to participants with a frequency change every 10 seconds (falling and rising tones between 500 Hz and 8000 Hz). The manner of presentation enabled passive listening (hearing the sound without engaging with speaker) with both ears (Suzuki et al., 2002). During the fMRI scan, participants were instructed to lie with their eyes closed and listen to the binaural auditory stimuli consisting of warble tones. The warble tones

were randomly presented to the participant, with five frequencies being used for ten seconds per frequency.

As shown in **Table 18** the warble tones were randomly presented to the participant, with five frequencies namely 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, and 8000 Hz being used for ten seconds per frequency.

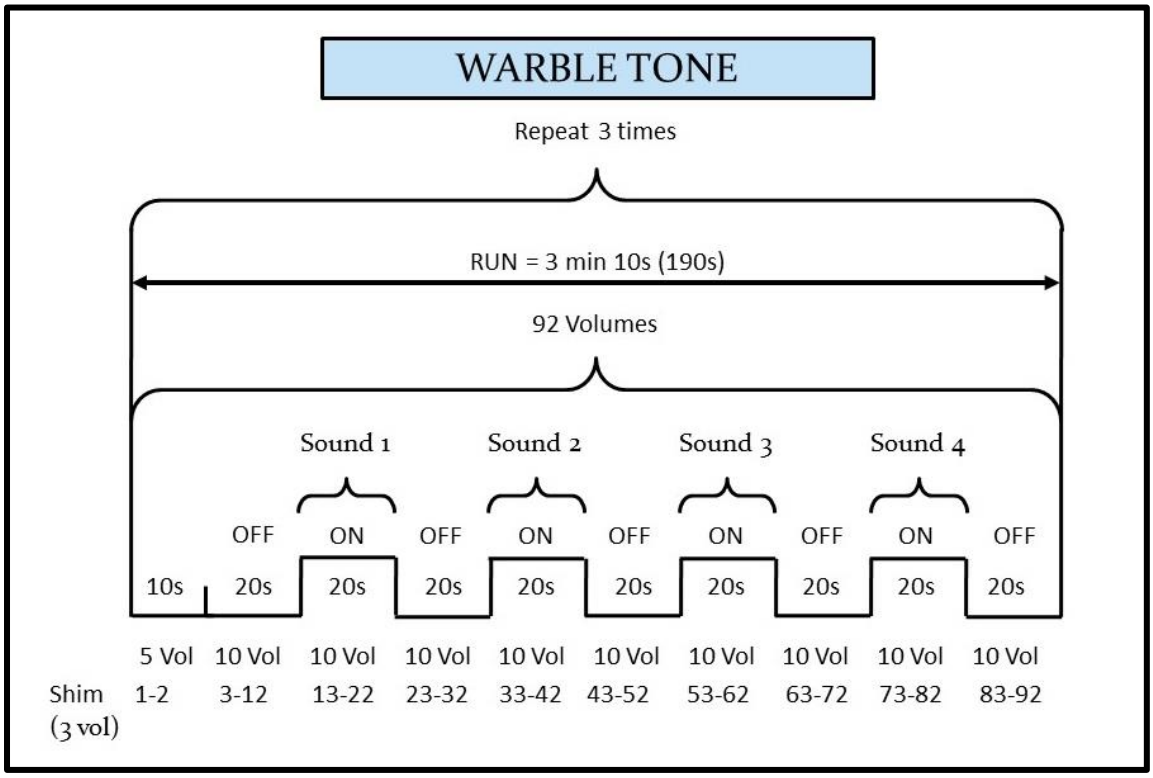


Figure 15: Warble tone task design

The number of experimental sessions and the volumes acquired per session are shown in Figure 15. During the auditory fMRI scan, participants were instructed to lie with their eyes closed and to listen to the binaural auditory stimuli comprising of 'warble tones'. Two ten seconds blocks (ten seconds per frequency total twenty seconds) of warble tones were alternated with twenty seconds blocks of silence during the fMRI scan (see Figure 15).

3.8.2. Participant preparation

All scans were acquired using a highfield MR scanner namely 1.5T Magnetom Espree Syngo MR B17 (Siemens, Erlangen, Germany). Plastic tubes 2.4 m in length and 10 mm in diameter delivered the auditory stimuli to the earphones which covered both ears of the participants. The plastic tubes were connected to an acoustic generator that was placed at the bale end of the MR scanner. The auditory stimuli with a sound pressure level of (95 dB) were presented from a digital music player outside the scanner room but the cables were connected to the acoustic generator and the plastic tubes connected to the Siemens headphones. Earphones were fitted on the participant's head to cover both ears for the auditory stimuli that were presented to both ears. A structural scan was acquired as well as two functional MRI (fMRI) acquisitions. During the fMRI scan, participants were instructed to lie with their eyes closed and listen to the binaural auditory stimuli which consisted of a set of nonsense syllables and a set of warble tones. The mean sound pressure level (SPL) of monosyllables was adjusted to 95 dB SPL at the inserted earphone, with calibration using a 1-kHz pure tone (prepared by Richard H. Wilson, PhD, Senior Research Career Scientist Audiology, Medical Center Mountain Home, Tennessee, in 2010 and available on CD-ROM).

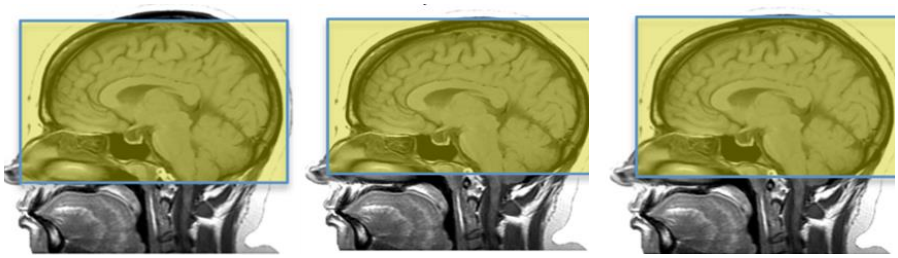
3.8.3. Data acquisition: Magnetic Resonance Imaging scanning protocol

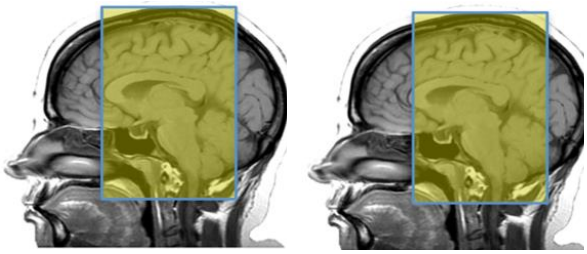
The 3D volume of an fMRI scan of the head is imaged every one or two seconds. This produces hundreds or thousands of completed images per scanning session. These data are information about the anatomy of the brain, and the data are used for processing and analysis using algorithms and statistical methods (Chen & Li, 2012). The MRI acquisition parameters are summarised in Table 19. All scans were acquired using the head coil HE.

Data collection was done with the following image properties:

- Pulse sequence type (gradient/spin echo, EPI/spiral)
- Field of view, matrix size, slice thickness, interslice skip
- Acquisition orientation (axial, sagittal, coronal)
- Order of acquisition of slices (sequential or interleaved)
- TE/TR/flip angle (Heuttel et al., 2009)

Table 19: MRI acquisition parameters

	t1_mpr_tra_iso	gre_field_mapping	ep2d_bold_moco_NS
	Pulse sequence type	**Pulse sequence type**	**Pulse sequence type**
	Tfl	gradient	ep2d_bold
	Area of acquisition	Area of acquisition	Area of acquisition
Area of Acquisition			
Field of view (mm)	250	250	250
matrix	256 x 256	64 x 64	64 x 64
Slices	1 slab (176 slices)	28	28
slice thickness (mm)	1	4	4
acquisition orientation	Axial	axial	axial
phase encoding direction	R-L	A-P	A-P
Order of Acquisition of slices	sequential, ascending	interleaved	interleaved
TE	3.02ms	(1) 4.76 (2) 9.52	30ms
TR	1650ms	513ms	2000ms
flip angle	15°	60°	80

	gre_field_mapping	ep2d_bold_moco_warbl
	Pulse sequence type	**Pulse sequence type**
	gradient	ep2d_bold
	Area of acquisition	Area of acquisition
Area of Acquisition		
Field of view (mm)	250	250
matrix	64 x 64	64 x 64
Slices	28	28
slice thickness (mm)	4	4
acquisition orientation	coronal	coronal
phase encoding direction	R-L	R-L
Order of Acquisition of slices	interleaved	interleaved
TE	(1) 4.76 (2) 9.52	30ms
TR	513ms	2000ms
flip angle	60°	80°

Key references for Table 19:

mpr: multiplanar reformat

iso: isotropic voxels same size in all three orthogonal axes

gre: gradient echo basic pulse sequence in which signal is dephased and refocused by gradient only, with a very short TR

ep (epi): rapid changes of frequency-encoding, fastest method of acquiring MRI k-space data

bold: blood oxygen level dependent, reduction of T2* in tissues due to build-up of deoxhaemoglobin during high oxygen demand

TE: echo time

TR: repetition time

(Adapted from ReviseMRI.com, 2013)

3.8.4. Processing of fMRI data (Pre-processing)

Pre-processing describes the procedures applied to fMRI data prior to the statistical analysis thereof this usually begins with converting the data from original MR format (DICOM) to a file format that is more manageable for use by the analysis software. The analysis software used in this case is BrainVoyager QX (Brain Innovation, Maastricht, The Netherlands). BrainVoyager is written in C++ to optimise speed, and it uses a 3D graphics background (Ashby, 2011). BrainVoyager is commercial software and requires a HASP USB dongle (James et al., 2014). BrainVoyager was used in this fMRI study because it is fast and user friendly. This software also performs automatic brain segmentation, surface reconstruction, cortex inflation, and flattening. In addition, BrainVoyager supports advanced data analysis of fMRI brain images in the Open Graphics Library. It uses cross-language for 2D, and 3D graphics implementing BrainVoyager software for rendering 3D computer graphics (surface module) (James et al., 2014).

- **Pre-processing of the FMR (Functional MR) project**

Loading the raw functional data and converting it into FMR data format created a functional MR (FMR) project. FMR was the 2D visualization of functional data. BrainVoyager supports various types of data formats including DICOM, ANALYZE, and PHILIPS_REC. The MR scanner names the DICOM files in a complex way. Before creating the FMR project, BrainVoyager renames the DICOM files using header information. This is done to avoid problems during importing and further analysis of the data (James et al., 2014). Pre-processing was used to improve the image quality by suppressing undesired distortions or enhancing some image features to be more suitable for further processing of the images. Pre-processing of functional data comprises several steps including mean intensity adjustment, slice scan time correction, 3D motion correction, spatial smoothing, and temporal filtering (James et al., 2014). Three dummy images were acquired in each run, and these were excluded from the analyses. Images were motion-corrected relative to the first volume of the functional run with trilinear/sinc interpolation. Images were corrected for different slice acquisition times and linear trends, spatially smoothed using a Gaussian filter (FWHM 4.0 mm), and temporally smoothed with a high pass filter of two cycles per point. All data exceeding the movement criterion of a 3.0 mm displacement or 3.0 degree rotation within a functional run was rejected (Heuttel et al., 2009).

- **Anatomical Data Processing**

The anatomical data was loaded into BrainVoyager and converted into BrainVoyager-supported VMR data format. VMR is the 3D graphical representation of anatomical data. This data may exhibit inhomogeneous intensity which can be removed by using the intensity inhomogeneous correction tool in BrainVoyager software (James et al., 2014). Before FMR–VMR alignment, the resolution of the VMR data set needed to match the resolution of the functional data. In order to do this, the VMR data set is iso-voxel to a resolution of 1.0 x 1.0 x 1.0 mm (James et al., 2014)**.**

For multi-subject analysis, the data set was transformed into a standard space. BrainVoyager provides two options for Talairach transformation: manual and automatic. In manual Talairach transformation, the user needs to find anterior commissure (AC), posterior

commissure (PC), anterior point of the cerebrum (AP), posterior point (PP), superior point (SP), inferior point (IP), most right point (RP), and most left point (LP) manually by dragging the mouse pointer to the specified region or entering the coordinate values.

In automatic Talairach transformation, the Talairach reference points (see above) are set automatically by the software. The Talairach transformation was needed for both anatomical and functional data for only multi-subject analysis (James et al, 2014). Each participant's functional data sets were co-registered to his/her high-resolution anatomical MRI, rotated into the AC-PC plane, and normalised to the Talaraich space using a linear transform calculated on the anatomical images. The 3.9 x 3.9 x 4.0 mm fMRI voxels were interpolated during Talairach normalisation to 3.0 x 3.0 x 3.0 mm (Heuttel et al., 2009).

- **Inter-modality registration**

Co-registration allows alignment of functional and anatomical data for the purpose of overlaying the brain activity precisely. Co-registration was done in two ways namely Initial alignment (IA) and Fine-tuning alignment (FA). This is the initial alignment and fine-turning alignment step of functional (FMR) and 3D anatomical data (VMR) co-registration

The initial alignment brings the functional and anatomical data into the same orientation, using fine-turning alignment rectified head movements. FMR data was set as the "source" and VMR data was set as the "target." FMR data is fixed and the VMR data is scaled, translated, and rotated with respect to FMR data (James et al., 2014).

3.8.5. Preparing fMRI data for statistical analysis

This section describes the procedures applied to prepare fMRI data prior to the statistical analysis.

- **The creation of the stimulation protocol**

Stimulation protocol allows defining the conditions (block or event-related design) used for the presentation of the stimuli. BrainVoyager saves this data as a protocol (PRT) file.

The same protocol file can be used for different FMR projects, if the same conditions are used for different subjects (James et al., 2014).

- **Statistical analysis of functional data**

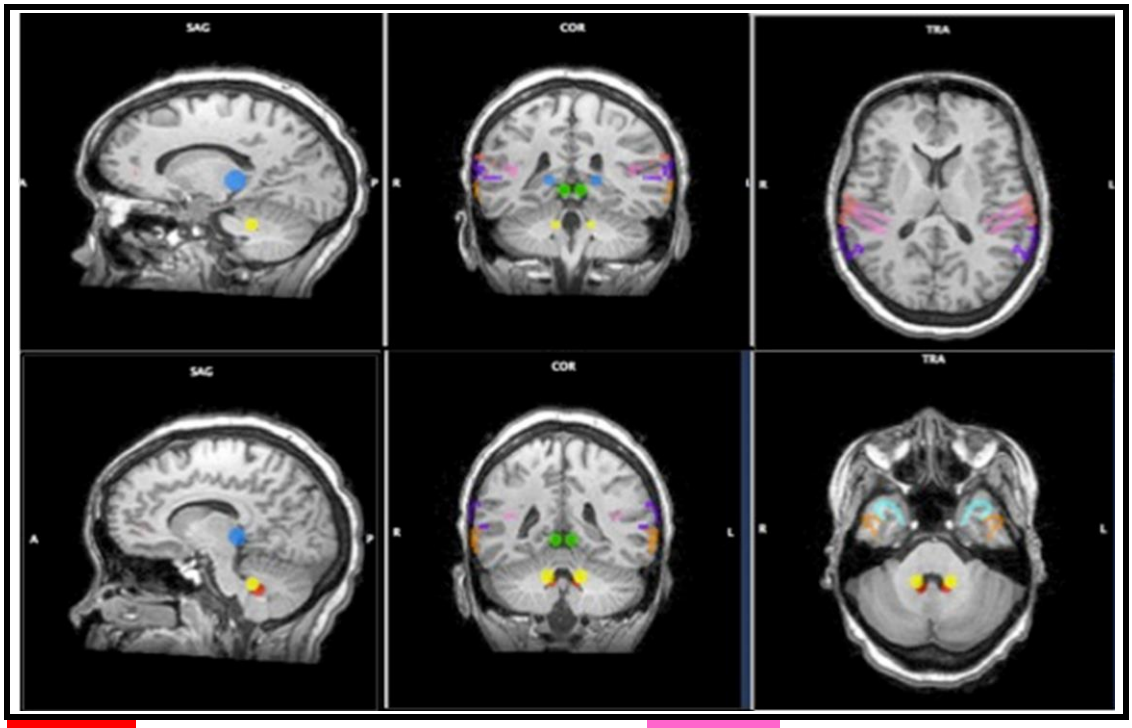
The statistical analysis test was performed to determine which voxels in the brain were significantly activated by a certain type of stimulus (auditory stimuli were used in this study). BrainVoyager software provides options for single subject and multi-subject statistical analysis. This software uses GLM for single subject analysis specifying statistical models. It is obtained by adding several explanatory variables known as predictors, which give precise activations. GLM analysis is a univariate method performed independently on each voxel. Time course and beta values are estimated for each voxel (Goebel, 2012).

Multi-subject data are analysed by using fixed-effects group analysis and random-effects group analysis. BrainVoyager also supports multivariate approaches like independent component analyses and multivoxel pattern analysis. The multivoxel pattern analysis tools include multivariate searchlight mapping and a machine learning classifier, the *support vector machine* (SVM), for analysing patterns in ROIs and for discriminating patterns that are potentially spread out across the whole brain. The end result of the statistical analysis is a statistical map that shows which voxels are significantly activated given a specified statistical threshold. The "threshold" statistical map can be overlaid directly to the functional (FMR) data, co-registered anatomical [volumetric MR (VMR)] data, or surface module for visualization (James et al., 2014).

Whole brain group analyses were performed for each task separately, with a random effect analysis of variance using the general linear model with predictors based on the known experimental blocks convolved by the standard hemodynamic function. The six motion correction parameters were added as predictors of no interest. For each task, beta maps were created for each participant to show the contrast of interest (auditory stimulus – baseline), and analysed at the second level using a repeated measures ANOVA, with one within-subjects factor (nonsense syllables or warble tones) and one between-subjects factor (HIV/AIDS group and control group) (Goebel, 2012).

A cluster size correction was used because the auditory pathway regions are very small, and the primary auditory cortex is also quite small (1-4 cm^3) (Hall, Haggard, Akeroyd, Palmer, Summerfield, Elliott, Gurney & Bowtell, 1999). In the whole brain analysis, the nonsense syllables generated a greater and more extensive response than the warble tones. The active regions were reported for the nonsense syllables if their voxelwise threshold extent was greater than **50 contiguous voxels** with a **FDR corrected p < 0.05**, where voxel size refers to the 1.0 x 1.0 x 1.0 mm resolution of the iso-voxeled structural images (Hall et al., 1999). In the whole brain analysis, the response to warble tones was less extensive than for the nonsense syllables. The active regions were reported for the warble tones if their voxelwise threshold extent was greater than **150 contiguous voxels** with a **FDR corrected p < 0.05**, where voxel size refers to the 1.0 x 1.0 x 1.0 mm resolution of the iso-voxeled structural images (Hall et al., 1999).

A priori **regions of interest (ROIs)** for the auditory pathway (see Figure 16) were defined, based on the study of structural brain changes in tinnitus by Mühlau, Rauschecker, Oestereicher, Gaser, Röttinger, Wohlschläger, Etgen, Conrad & Sandér (2005). ROIs were defined as spheres with radii of 5.0 mm (8.0 mm for the medial geniculate nucleus), centred on the MNI co-ordinates for the ventral and dorsal cochlear nuclei (±10, –38, –45), superior olivary complex (±13, –35, –41), inferior colliculus (±6, –33, –11), medial geniculate nucleus (±17, –24, –2), and the primary and secondary auditory cortices corresponding to Brodmann areas 41, 42, and 22. The cortical areas were defined based on the Talairach brain atlas (Talairach & Tournaux, 1988) in BrainVoyager. The MNI (Montreal-Neurological Institute) coordinates were transformed into Talairach coordinates using Brainmap (http://www.brainmap.org/ale/). Separate analyses for participants were performed on the average signal in each ROI, using the general linear model. The beta values generated by this analysis, which reflected the mean percent signal change in each region for each condition for each participant, were used to calculate signal change during hearing of nonsense syllables/warble tones compared with the silence task (http://BrainVoyager.com/ index.html) (Goebel et al., 2012). Group comparisons were performed using a one-tailed *t*-test.



	CNR	cochlear nucleus right		Brodmann - Area - 41
	CNL	cochlear nucleus left		Brodmann - Area - 42
	SOCR	superior olivary complex right		
	SOCL	superior olivary complex left		
	ICR	inferior colliculus right		
	ICL	inferior colliculus left		
	MGNR	medial geniculate nucleas right		
	MGNL	medial geniculate nucleas left		

Figure 16: Regions of interest of the auditory pathway

Activations for each of the tasks were examined separately in the control and exploratory/experimental (HIV/AIDS) groups, and regions where activation differed between the groups were also identified. For the nonsense syllables task, the voxelwise threshold was set to $p < 0.05$ (corrected for multiple comparisons using the False Discovery Rate method).

3.9. fMRI statistical analysis tests used

A statistical analysis method currently used in fMRI research combines the traditional p-value threshold with a required minimum number of contiguous voxels (commonly referred to as a 'cluster size threshold'). This assumption is based on the facts that functional imaging data are spatially correlated, and that true positive activation tends to occur in clusters of contiguous voxels (Gross & Binder, 2014). Statistical methods are a helpful tool to interpret the information from neural imaging data into statistical data (Chen & Li, 2012).

a) A spreadsheet was created in Microsoft Excel® to combine all the data obtained from all the participants, the ROIs, and the whole brain activation areas so that they could be imported into a spreadsheet in the statistical analysis program SPSS (IBM SPSS Statistics for Macintosh Version 23.0) (Schwartz, 2014)

b) The **independent sample test (t-test)** this test of significance evaluates the differences between the means obtained from the two groups. The two groups (control and HIV/AIDS) were evaluated with the difference of means of the ROIs areas in both tasks (nonsense and warble task). Separate analyses for participants were performed on the average signal in each ROI (see Figure 16) using the general linear model (Schwartz, 2014)

c) A **one-tail** is a statistical test in which the criteria area falls under one side of the normal distribution, a specific difference between groups**, unpaired *t*-test** was used to examine the significance of differences between the means of continuous variables between two groups of the ROIs. It was also used to determine the p-values. If the p-value was smaller than a predetermined alpha level (0.05) it was considered to be statistically significant (D'Abramo, Zingaropoli, Oliva, D'Agostino, Al Moghazi, De Luca, Iannetta, d'Ettorre, Ciardi, Mastroianni&Vullo, 2016).

d) **UNIANOVA** procedure provides a regression analysis and analysis of variance for one dependent variable by one or more variables / **one-way within groups ANOVA is the same as UNIANOVA** it is an analysis of variance that is used when a design has one independent variable at a level of that variable that have been experienced by all the participants and the researcher has a hypothesis about the difference among the means associated with each level of independent variable (Schwartz, 2014)

In this study between the participant an unianova was done to determine if gender, age and education played a differentiating role in r-square and linear regression (CD4 count and years on ART) and ANOVA was also performed to examine associations between brain activation and percentage signal change during presentation of auditory stimuli (ROI) areas were used (Schwartz, 2014).

- **R-square (coefficient of determination)** indicates the "goodness of fit" to illustrate how close the data points fit the line on the scatter plot (Schwartz, 2014).

- **Linear regression** estimates the straight line that best fits a scatterplot of the two variables. The equation for that line can be used to predict values on the criterion variable (Schwartz, 2014).

- **Analysis of variance (ANOVA)** is a collection of statistical models used to analyse the differences among groups' means. It was used to test the degree to which the results of the groups varied or differed. To detect specific differences when groups were found to be significantly different ($p<0.05$), pairwise comparisons between groups were done according to Fisher's exact test. Given a null hypothesis and a significance level, the corresponding F test rejects the null hypotheses if the value of the F statistic is large (Le Prell et al., 2007). The p value is understood as the probability that a null hypothesis was proven to be true. T-tests to determine significant differences between the mean scores of two groups were also used to determine p- values. If the p value was smaller than a predetermined alpha level of 0,05 for this study it was considered to be statistically significant, and the null hypothesis was rejected, and the alternative hypothesis accepted (Brewer & Stockton, 2010).

The t-test used the groups (Control and HIV/AIDS) in all the ROIs and the BA41 and BA42 was significant.

3.10. Trustworthiness of the current quantitative research project

Research is only as good as the trustworthiness of the data that was used. The efforts made to enhance the trustworthiness of the study are set out in Table 20.

Table 20: Strategies used to enhance trustworthiness of the study

Strategy	Type	Technique	Application of the technique in this study
Validity "Validity refers to the extent to which an empirical measure adequately reflects the real meaning of the concept under consideration" (Babbie, 2013). The word "validity" can have several different meanings, the most essential meaning is probably "appropriateness". A valid measure is measuring or testing what it is suppose to measure or test.	**Content validity** The extent to which the items of the measurement instrument reflect the theoretical content domain of the construct being investigated	• Define the specific construct and specify the theoretical content areas that it implies • Determine items relevant to specific content areas • Select most representative items of each content area	Literature study was done to determine the current and most recent status and determine the extent of information and results.
	Construct validity "The degree to which a test (questionnaire) measures the theoretical construct or abstract it was intended to measure" (Struwig & Stead, 2001:141)	Pilot study	Pilot study was done to help with the validity of the CANS sound in normal hearing adults with and without HIV/AIDS, and to ensure the researcher's acquaintance with skills to interpreting the results of these measurements accurately.
	Criteria related validity The extent to which the results of a measurement instrument are compared to another independently valid criterion	Comparison to existing theoretical criteria	Al literature study was done to determine the current and most recent studies of normal findings in fMRI and determine the extent of the information and results.

Strategy	Technique	Application of the technique in this study
Reliability "an instrument is reliable to the extent that independent administrations of it or comparable instrument consistently yield similar results" (De Vos & Fouché, 1998:85) Reliability of measures is very important in a research study, for the reason that if the measures are not reliable the study cannot produce valuable information. Reliability refers to the uniformity and ability of an instrument to obtain the same results every time it is performed (French et al., 2001).	• Clearly conceptualise all constructs • Use of multiple indicators of variable • Use of a pilot study	• Developing unambiguous, clear theoretical definitions for all constructs. Care was taken to ensure that each measure indicated one and only one concept. • Two or more indicators of the same construct were used • A pilot study was conducted to determine the suitability and effectiveness of the data collection instruments and procedures.
Objectivity Known as confirmability in qualitative research	Independent judgement of quantitative procedures	The researcher was trained to use the instrument, so that the same results could be obtained for each use (Leedy & Ormrod, 2010)

3.11. Conclusion

The research aims, research design, material, apparatus, and methods used in this research project are described in this chapter. Information about the complete research process is provided, including the formulation of the research question, sample selection and description, material and apparatus that were used as data collection tools, and the methods used for data collection and analyses. The results, and a discussion of the results, are presented in the chapters that follow.

CHAPTER 4

RESULTS

"Change is the end result of all true learning"

(Leo Buscaglia)

4.1. Introduction

In this chapter the numerical results obtained from the data are presented according to the main aim and sub-aims as set out in the methodology section. The main aim of the study was to determine the response of the CANS to sound in normal hearing participants with and without HIV/AIDS. To facilitate navigation through the results, the main aim and sub-aims of the research are described in Figure 17.

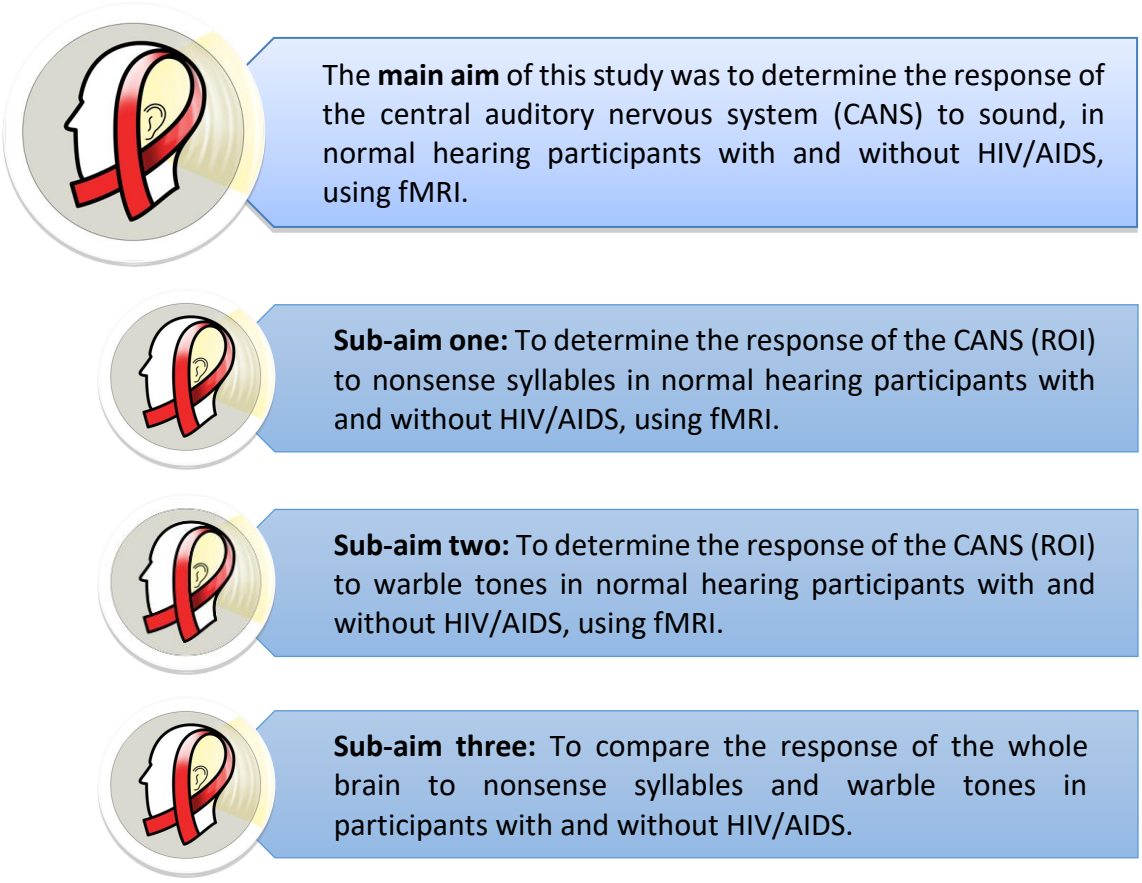


Figure 17: Summary of the main aim and sub-aims of the study

With regard to the statistical analysis of the different sub-aims, the following aspects must be kept in mind. For the purpose of sub aim 1 and sub-aim 2 the ***mean percentage signal change in the ROI*** indicates the **mean value of voxels** that are calculated at that specific time of point for each of the defined ROIs (spheres) namely the CN, SOC, IC, MGN, BA41 and BA42. For sub aim 3, the auditory fMRI data was not limited to the ROIs, but included a voxel-wise analysis of the whole-brain[3] area which was performed in order to evaluate the multiple areas of activation and responses to **nonsense syllables** and **warble tones.**

4.2. Results of sub-aim one

Sub-aim one: To determine the response of the CANS (ROI) to nonsense syllables in normal hearing participants with and without HIV/AIDS, using fMRI.

In order to explore the data of the response of the CANS to **nonsense syllables**, it was necessary to perform a **region of interest (ROI)** analysis of the CANS areas in the brain.

The presentation of the results will **firstly** focus on the defined anatomical ROIs areas (CN, SOC, IC, MGN, BA41 and BA42) for which mean values for all voxels in the particular ROI area were calculated at a given point in time. **Secondly**, the results will be presented and compared according to the possible confounding influences such as gender, age, and education. **Thirdly,** the effect of the CD4 count and ART on the neural response of CANS (ROIs) to auditory stimulation in the HIV/AIDS group will be presented.

The response of the CANS (ROIs) to nonsense syllables

In Table 21 the mean percentage signal change during the nonsense syllables in the defined ROIs are presented for both groups of participants.

[3] Whole brain: The analysis of the whole brain was performed on each voxel, whereas for the ROI analysis only the voxels inside the ROI are used (Poldrack, 2007)

Table 21: Mean percentage signal change during nonsense syllables in defined ROIs

Area	Group	n	Mean	Std Deviation	*p*
cochlear nucleus right	HIV/AIDS	12	0.008	0.034	0.40
	Control	15	0.005	0.028	
cochlear nucleus left	HIV/AIDS	12	0.003	0.038	0.16
	Control	15	-0.013	0.046	
superior olivary complex right	HIV/AIDS	12	0.003	0.031	0.40
	Control	15	0.000	0.039	
superior olivary complex left	HIV/AIDS	12	0.001	0.029	0.27
	Control	15	0.011	0.048	
inferior colliculus right	HIV/AIDS	12	0.022	0.094	0.19
	Control	15	-0.004	0.053	
inferior colliculus left	HIV/AIDS	12	0.021	0.083	0.12
	Control	15	-0.008	0.046	
medial geniculate nucleus right	HIV/AIDS	12	-0.001	0.014	0.32
	Control	15	-0.005	0.023	
medial geniculate nucles left	HIV/AIDS	12	-0.005	0.016	0.48
	Control	15	-0.005	0.022	
BA 41	HIV/AIDS	12	0.107	0.054	0.03*
	Control	15	0.154	0.068	
BA 42	HIV/AIDS	12	0.068	0.049	0.03*
	Control	15	0.1317	0.103	

*Note. $p \leq 0.05$ significant difference

Both groups of participants showed activation in all the ROIs, however, there is no significant difference in CN, SOC, IC, MGN between participant groups but the mean percentage signal change in BA41 (anterior transverse temporal area) and BA42 (posterior transverse temporal area) differed significantly between the HIV/AIDS and control groups.

The activation (mean percentage signal change) in both hemispheres for the areas BA41 and BA42 is presented in Figure 18.

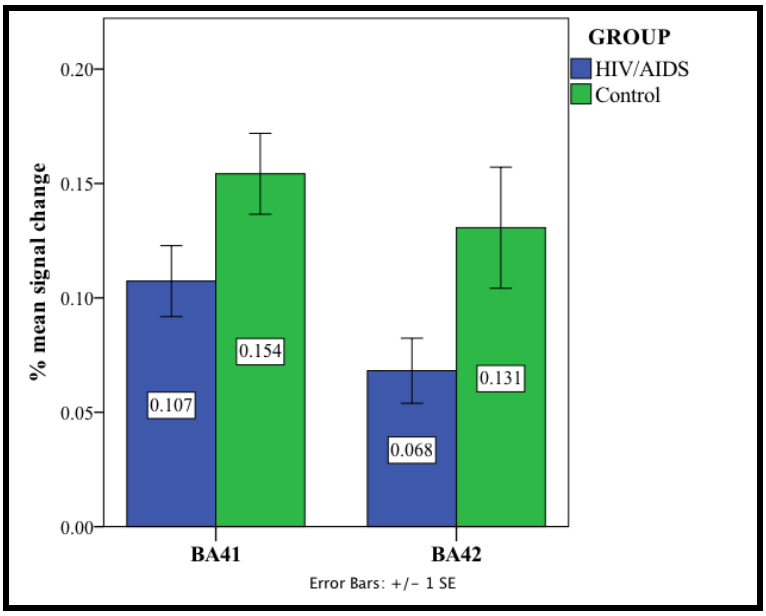


Figure 18: Mean percentage signal change during the nonsense syllables task in BA41 and BA42

It is clear that the control group showed a higher mean percentage signal[4] change in both BA41 (M = 0.154) and BA42 (M = 0.131) than the HIV/AIDS group which showed a mean percentage signal change of 0.107 in BA41 and 0.068 in BA42. These obvious differences were compared using a one-tailed t-test (See table 22).

Table 22: Difference between the groups for BA41 and BA42 as determined by the one-tailed t-test

Area	Group	n	Mean	Std Deviation	Std Error Mean	95% CI for Mean Difference		t	df	*p*
						lower	upper			
BA 41	HIV/AIDS	12	0.107	0.537	0.015	-0.967	0.002	-1.9	25	0.03*
	Control	15	0.154	0.068	0.017					
	Total	27	0.133	0.066	0.013					
BA 42	HIV/AIDS	12	0.068	0.049	0.014	-0.129	0.004	-1.9	25	0.03*
	Control	15	0.131	0.103	0.026					
	Total	27	0.103	0.088	0.017					

*Note. $p \leq 0.05$ significant difference

[4]Signal – Comparing the signal at voxel against a baseline average of the signal taken from the entire brain (Poldrack, 2007)

As evident from Table 22 the mean percentage signal change (mean value of voxels in the ROI areas) differed significantly between the two groups. There was a statistically significant difference between the HIV/AIDS group and control group in the activation of BA41 [$t(25) = -1.96$, $p = 0.03$] as well as in the activation of BA42 [$t(25) = -1.13$, $p = 0.03$].

The mean percentage signal change (*M*) in the control group is significantly (at 5% level) higher ($M = 0.154$) than the mean percentage signal in the HIV/AIDS group ($M = 0.107$). For the other variable namely BA 42 the mean percentage signal change (*M*) in the control group is once again significantly (at 5% level) higher ($M = 0.131$) than the average in the HIV/AIDS group ($M = 0.068$).

Influence of gender, age and education on the mean percentage signal change in BA41 and BA42

The influence of possible confounding factors such as gender, age, and education on the mean percentage signal change in BA41 and BA42 was determined for the two groups. Since it has been shown that auditory fMRI results may be influenced by **gender** (Ramjee & Daniels, 2013), **age** (Hakkers, Arends, Barth, Du Plessis, Hoepelman & Vink, 2017), and **education** (Ernst, Chang, Jovicich, Ames & Arnold 2002), it was important to establish if these effects were also noted in the current data. Table 23 presents the results of the one-way between-participants Analysis of Variance (ANOVA) for the mean percentage signal change in BA41 for gender, age and education.

Table 23: Influence of gender, age and education on the level of activation in BA41

Area BA 41	df	Mean Square	F	*p*
Effect of **gender** (male = 9, female = 18)	1.00	0.02	4.65	0.04*
Effect of **age** (21-30 = 18, 31-40 = 6, 41-50 = 3)	1.00	0.00	0.00	0.95
Effect of **education** (matric = 11, post matric = 16)	1.00	0.00	0.18	0.68

* Note. Significant difference at the $p \leq 0.05$ level

The results in Table 23 indicate that age ($p = 0.95$) and education ($p = 0.68$) had no statistically significant effect on the mean percentage signal change in BA41. However, gender had a significant effect at the 5% level of statistical significance for BA41 with a p-value of

0.04. The mean percentage signal change with regard to gender was further investigated by means of descriptive statistics in order to determine the difference between the two groups.

Table 24 displays the results of the detailed one-way ANOVA test to determine whether there is statistical evidence that the means (according to gender) for the two groups are significantly different for BA41.

Table 24: Effect of gender on the activation in BA41 for the two participant groups

Gender	**Group**	**n**	**Mean**	**Std. Deviation**	
Male	HIV/AIDS	4	0.146	0.075	
	Control	5	0.123	0.057	
Between participants		**df**	**Mean Square**	**F**	**Significant**
Male * Group Interaction		1	0.001	0.278	**0.614**
Gender	**Group**	**N**	**Mean**	**Std. Deviation**	
Female	HIV/AIDS	8	0.088	0.029	
	Control	10	0.170	0.071	
Between participants		**df**	**Mean Square**	**F**	**Significant**
Female * Group Interaction		1	0.030	9.342	**0.008***
Between participants		**df**	**Mean Square**	**F**	**Significant**
Gender * Group Interaction		1	0.016	4.645	**0.042***

*Note. significant difference at the $p \leq 0.05$ level

The results in Table 24 of the independent variables namely gender and group, show that there was no significant difference between the group as a whole and male participants (p=0.614), but a significant difference between the group and female participants (p=0.008) for the area BA41. The interaction between the participants' gender and the group has a statistically significant effect ($p = 0.042$) on the dependent variable BA41. Figure 19 indicates that the difference between the gender groups mean (male and female) depended on their HIV/AIDS status and the difference between the mean for the HIV/AIDS and the control group depended on their gender.

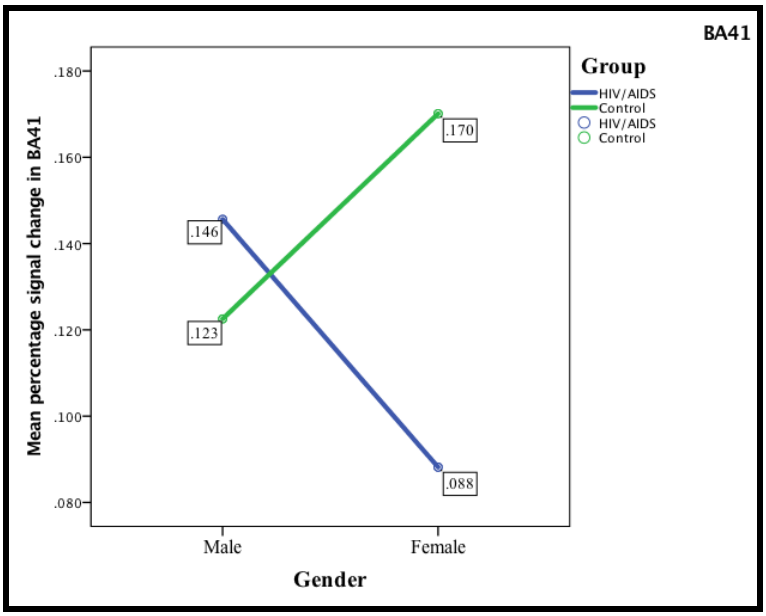


Figure 19: Difference between the mean percentage signal change in BA 41 for the HIV/AIDS and control groups

In Figure 19 the interaction between gender (male and female participants) in the two groups (HIV/AIDS and control) showed a difference. The difference between the two groups depended on gender: in the HIV/AIDS group the mean percentage signal change in males (M = 0.146) was greater than in females (M = 0.088), while in the control group the mean percentage signal change in the males (M = 0.123) was smaller than in females (M = 0.170).

To further investigate the response of the CANS (ROIs) to nonsense syllables, the signal changes in the area of **BA42** (see Table 25) were statistically analysed to determine if gender, age, and education affected the results for all the participants.

Table 25: Influence of gender, age and education on the level of activation in BA42

Area BA 42	df	Mean Square	F	*p*
Effect of **gender** (male = 9, female = 18)	1.00	0.03	4.92	0.04*
Effect of **age** (21-30 = 18, 31-40 = 6, 41-50 = 3)	1.00	0.00	0.04	0.85
effect of **education** (matric = 11, post matric = 16)	1.00	0.00	0.00	0.99

* Note. Significant difference at the $p \leq 0.05$ level

The results in Table 25 indicate that age (p = 0.85) and education (p = 0.99) had no statistically significant effect on the mean percentage signal change in BA42. However, gender had a significant effect at the 5% level of statistical significance for mean percentage signal change for BA42, with a p-value of 0.04. The mean percentage signal change with regard to gender was further investigated by means of descriptive statistics in order to determine the difference between the two groups.

Table 26 displays the results of the detailed one-way ANOVA test to test for the effects between participants and gender in the two groups for BA42.

Table 26: Effect of gender on the activation in BA42 of the two participant groups

Gender	**Group**	**N**	**Mean**	**Std. Deviation**	
Male	HIV/AIDS	4	0.097	0.068	
	Control	5	0.065	0.086	
Between participants		**Df**	**Mean Square**	**F**	**Significant**
Male * Group Interaction		1	0.002	0.352	**0.572**
Gender	**Group**	**N**	**Mean**	**Std. Deviation**	
Female	HIV/AIDS	8	0.034	0.029	
	Control	10	0.098	0.071	
Between participants		**df**	**Mean Square**	**F**	**Significant**
Female * Group Interaction		1	0.053	9.07	**0.008***
Between participants		**df**	**Mean Square**	**F**	**Significant**
Gender * Group Interaction		1	0.029	4.917	**0.037***

*Note. significant difference at the $p \leq 0.05$ level

The results in Table 26 show no significant difference between the group and male participants (p=0.572), but a significant difference between the group and female participants (p=0.008) for the area BA42. The interaction between the participants gender and the group has a statistically significant effect (p = 0.037) on the dependent variable BA42. Figure 20 indicates that the difference between the gender groups means (male and female) depended on their HIV/AIDS status and the difference between the mean for the HIV/AIDS and control group depended on their gender.

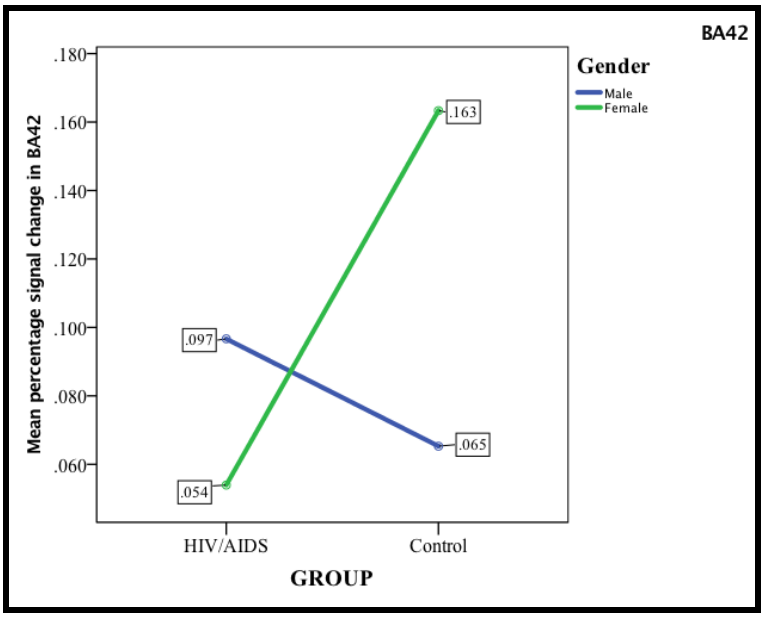


Figure 20: Difference between the mean percentage signal change in BA42 for the HIV/AIDS and Control groups

In Figure 20 the interaction between gender (male and female) participants in the two groups (HIV/AIDS and control) showed a difference. The difference between the two groups depended on gender: in the HIV/AIDS group the mean percentage signal change in males (*M* = 0.097) was greater than in and females (*M* = 0.034), while in the control group the mean percentage signal change in males (*M* = 0.086) was smaller than in females (*M* = 0.098).

The effect of CD4 count on the mean percentage signal change in BA41 and BA42

It was also considered relevant to determine whether the CD4 count had any effect on the neural response of the CANS (ROIs) to auditory stimulation in the HIV/AIDS group.

Figure 21 described the linear regression analysis between **CD4 count** and the mean percentage signal change in **BA41** and **BA42**.

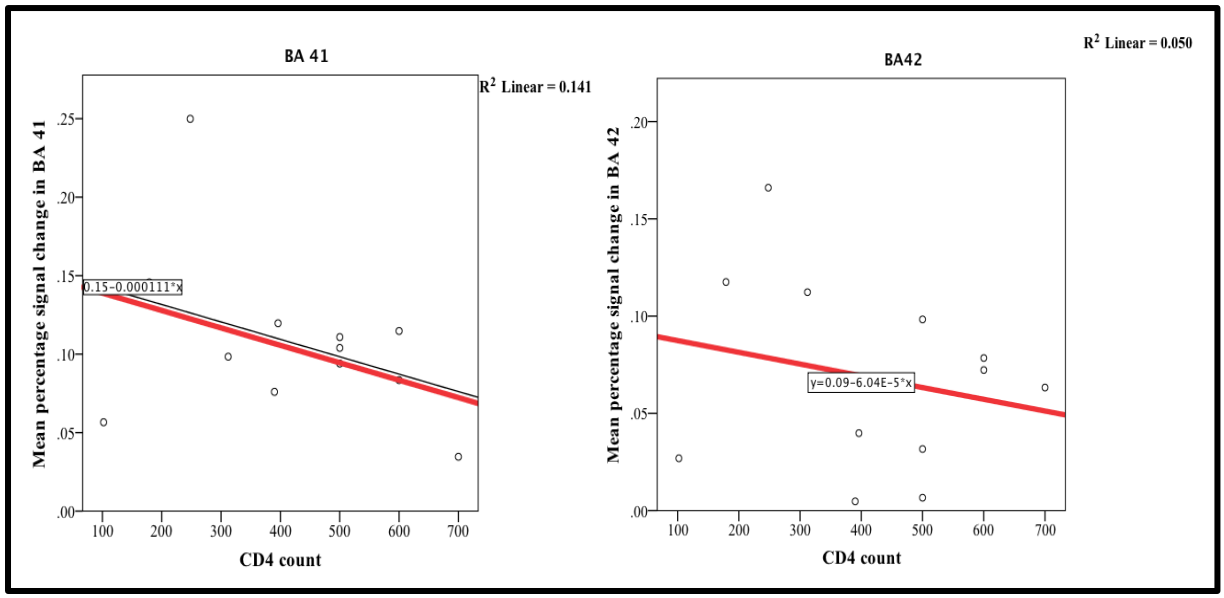


Figure 21: The linear regression between CD4 count and the mean percentage signal change in BA41 and BA42

From Figure 21 it is clear that the random effects of the linear regression model in SPSS show inverse correlations between the mean percentage signal change in both ROIs namely: BA41 and BA42, compared to the CD4 count. The slope of the regression line is negative and the value of y decreases (mean percentage signal change in BA41 or BA42) as x increases (CD4 count) (Powers & Xie, 2008). The variance of the data is explained by the fitted regression line, however a slight tendency was observed according to the R-squared values (Schwartz, 2014).

R-square is known as the coefficient of determination, since the R-square values indicate how close the data were to the fitted line of the regression model. This is also known as the *"goodness of fit"* of the regression model. The use of the statistical procedures estimates the straight line that best fits the scatter plot of the two variables and can be used to predict the values for those variables (Bowers, House, Owens & Bewick, 2013; Asby, 2011; Schwartz, 2014). The R-square values indicate how close the data (correlation between CD4 count and the mean percentage signal change in BA41 and BA42) are to the fitted regression line as indicated in Figure 21. The R–square is 0.141 for BA41, while the value is p =0.228 and the R–square is 0.050 for BA 42. The p value for BA41 is p=0.486, indicating that for BA 41 only 14% (a moderate to strong fit) and for BA 42 0.05 % (a weak fit) of the variance in the dependent variable is accounted for. The p-values indicate no significant correlation between

the CD4 count and the activity in BA41 and BA42 (see Figure 21). The HIV/AIDS disease severity in terms of the CD4 count and the mean percentage signal change in BA41 and BA42 area during the nonsense syllables task showed the following tendency: the higher the CD4 count in the HIV/AIDS group, the lower the activation of the signal change in either BA41 or BA42. Although this tendency was observed, it would require a bigger sample to shed more light on these findings.

The effect of ART on the mean percentage signal change in BA41 and BA42

The correlation between the years on ART and the mean percentage signal change in BA41 and BA42 of the participants with HIV/AIDS was determined by means of a linear regression model in SPSS. Figure 22 described the relation between the years on ART and the percentage signal change in BA41 and BA42 for participants with HIV/AIDS

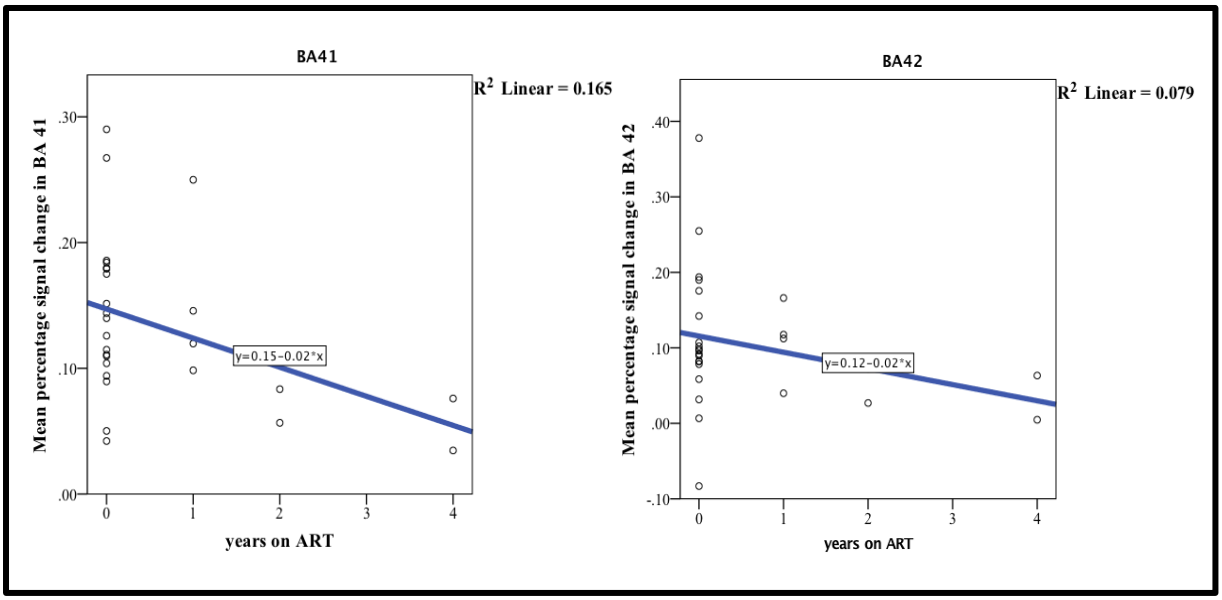


Figure 22: Relation between years on ART and mean percentage signal change in BA41 and BA42

It is clear that a negative coefficient was obtained, showing an inverse correlation between the years on ART and the activated BOLD signals in BA41 and BA42 areas during the nonsense syllables task. The random effects linear regression model in SPSS showed inverse correlations between the mean percentage signal change in BA41 or BA42 and ART use in years the slope of the regression line is negative and the value of y-axis decreases (mean

percentage signal change in BA41 or BA42) as x-axis increases (ART use in years) (Powers et al., 2008). Although this tendency was observed, it would require a bigger sample to shed more light on these findings.

4.3. Results of sub-aim two

Sub-aim two: To determine the response of the CANS (ROI) to warble tones in normal hearing participants with and without HIV/AIDS, using fMRI.

In order to explore the data concerning the response of the CANS to **warble tones**, it was necessary to perform a **region of interest (ROI)** analysis of the CANS areas in the brain.

The presentation of the results will focus on the mean percentage signal change calculated for each defined ROI (CN, SOC, IC, MGN, BA41 and BA42) for the two groups of participants (Table 27). The **one-tailed *t*-test** (Altman, 2001) was conducted to determine if the difference in activation in these ROIs between the two groups could be regarded as significant or not.

Table 27: Mean percentage signal change during warble tone task in defined ROI areas

Area	Group	n	Mean	Std Deviation	*p*
cochlear nucleus right	HIV/AIDS	12	-0.012	0.079	0.16
	Control	15	0.016	0.046	
cochlear nucleus left	HIV/AIDS	12	0.006	0.049	0.12
	Control	15	0.032	0.082	
superior olivary complex right	HIV/AIDS	12	-0.040	0.138	0.14
	Control	15	0.004	0.073	
superior olivary complex left	HIV/AIDS	12	0.036	0.097	0.38
	Control	15	0.041	0.139	
inferior colliculus right	HIV/AIDS	12	-0.046	0.086	0.07
	Control	15	-0.002	0.067	
inferior colliculus left	HIV/AIDS	12	-0.027	0.057	0.15
	Control	15	0.005	0.075	
medial geniculate nucleas right	HIV/AIDS	12	-0.004	0.032	0.43
	Control	15	-0.002	0.029	
medial geniculate nucleas left	HIV/AIDS	12	0.007	0.067	0.31
	Control	15	-0.005	0.034	
BA 41	HIV/AIDS	12	0.029	0.083	0.47
	Control	15	0.031	0.054	
BA 42	HIV/AIDS	12	0.065	0.158	0.17
	Control	15	0.029	0.061	

*Note. $p \leq 0.05$ significant difference

The only ROI where a borderline significant difference of activation occurred was in the **inferior colliculus** in the **right hemisphere** The control group showed a higher mean percentage signal change in the right **inferior colliculus** (*M* = -0.0022), than the HIV/AIDS group (*M* = -0.0458). An actual negative BOLD response is associated with a decrease in oxygen consumption and neural activity (Harel, Lee, Nagaoka, Kim & Kim, 2002).

From Table 27 it is clear that ROIs CN, SOC, IC, MGN, BA41 and BA42 have different mean percentage signal change values. There were no significant differences between all the ROIs, but there were some of the ROIs with positive mean percentage signal change such as the left CN, left SOC, BA41 and BA42.

Detailed results of the t-test are presented in Table 28.

Table 28: Results of the one-tailed t-test

Area	Group	n	Mean	Std Deviation	Std Error Mean	95% CI for Mean Difference		t	df	*p*
						lower	upper			
ICR	HIV/AIDS	12	-0.0458	0.094	0.027	-0.104	0.017	-1.48	25	0.07
	Control	15	-0.0022	0.053	0.014					
	Total	27	0.0258	0.074	0.015					

*Note. p ≤ 0.05 significant difference

The means and standard deviations are reported in Table 28. The control group (*M* = 0.0412, *SD* = 0.0526) and the HIV/AIDS group (*M* = -0.0217, *SD* = 0.0944) differed significantly (p = 0.07) in the right inferior colliculus area of the warble tones task.

These results can be visually described as follows:

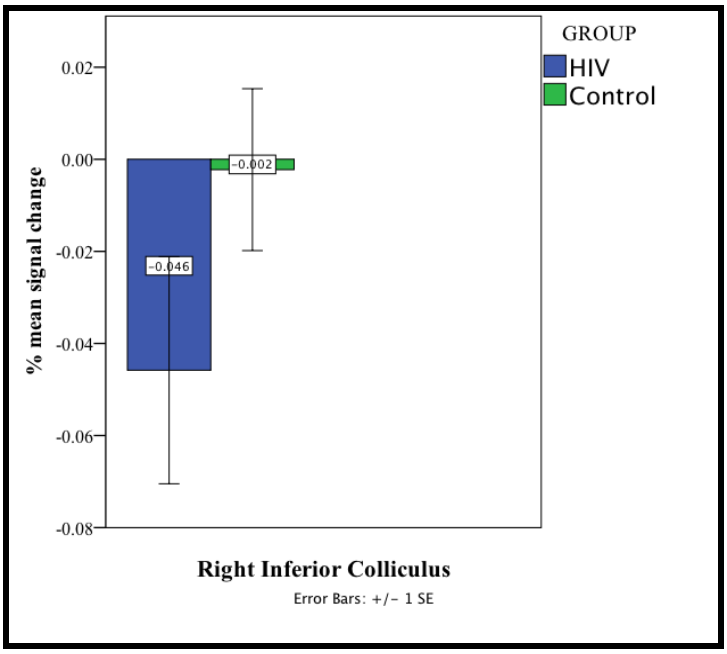


Figure 23: Mean percentage signal change during the warble tones task in the right inferior colliculus

The only ROI where a difference of activation occurred was in the **inferior colliculus** in the **right hemisphere** of the brain as shown in Figure 23. This negative response of the BOLD mean percentage signal change reflects a decrease in cerebral blood flow (decrease in oxygen consumption and neural activity) which can be induced by a reallocation of blood flow from the less demanding areas to the most cerebral blood flow demanding regions in the brain.

4.4. Results of sub-aim three:

Sub-aim three: To compare the response of the whole brain to nonsense syllables and warble tones in participants with and without HIV/AIDS.

The results of a whole brain analysis can provide significant information about the signal activation differences in the individual regions of the brain. When a group of voxels has been identified in the whole brain analysis as being potentially informative, the strategy for demonstrating whether that cluster is indeed informative is to create a ROI from that cluster and then characterise the properties of that ROI. If the ROI made from the cluster is shown to be informative, the researcher is justified in concluding that the cluster itself is informative relative to the results found in the whole brain (Etzel, Zacks & Braver, 2013).

In the current study, a whole brain analysis was done to determine and compare the multiple areas of activation and responses to nonsense syllables and warble tones. The results of the analysis will be presented as follows:

- Significantly increased brain activation during the sound tasks (nonsense syllables and warble tones) in the control group and HIV/AIDS group respectively, compared to the baseline[5]
- Group differences in significantly increased brain activation during the sound tasks (nonsense syllables and warble tones) between the control group and the HIV/AIDS group when compared to each other.

It should be noted that the results in this section were analysed by using voxel-by-voxel comparisons. Voxel-by-voxel comparison involves a voxel-wise comparison of the local concentration of grey or white matter of the brain in a local area in the brain between two groups of participants (Ashburner & Friston, 2000).

[5] Baseline: Baseline of the brain is the response of the brain in the absence of activation. The brain is never completely at rest (Klein, 2014).

These concentrations of grey or white matter in a local area are voxels[6] that represent clusters[7] in the brain. Spatially contiguous clusters of **voxels** that are **activated** together are considered of particular interest for analysis. Activation maps are based on these analysis units (Heller, Stanley, Yekutieli, Rubin & Benjamini, 2006).

This group clustering method depends on combining the data to the desired number of clusters. The whole brain clustering of data can be used in any combination from 50 to 1000 voxels, although it must be in multiples of 50 voxels (Craddock, James, Holtzheimer, Hu & Mayberg 2012). In the current study the activated areas were used with voxels above 1000 when groups were compared to baseline (Table 29 and Table 31) and the activated areas with voxels above 300 were used in group differences (Table 30 and Table 32).

Whole brain analysis of the response to nonsense syllables

In the whole brain analysis it was clear that the nonsense syllables generated a greater and more extensive response than the warble tones. As a result of this, the active regions were reported for the nonsense syllables if their voxelwise threshold extent was greater than **50 contiguous voxels** with a **FDR corrected $p < 0.05$**, where the voxel size refers to the 1.0 x 1.0 x 1.0 mm resolution of the iso-voxeled structural images (Hall et al., 1999).

In Table 29 and Figure 24 the regions that show *significantly increased brain activation* ($p < 0.05$) during the nonsense syllables task, compared to *baseline*, are summarised for the **control group** and **HIV/AIDS group** respectively. The voxel by voxel comparison of the activated areas with voxels above 1000 will be used and noted.

[6] Voxel: A voxel is a unit of graphic information that represents the activity of a particular coordinate in three-dimensional space (Poldrack, 2007).
[7] Clusters: Clusters are defined as contiguous volumes of voxels (Heller et al., 2006).

Table 29: Regions showing significantly more activity during the nonsense syllables task compared to baseline (voxelwise threshold of 50 contiguous voxels)

Region	Brodmann area	Mean *x*[a]	Mean *y*[a]	Mean *z*[a]	Number of voxels[b]	Max *t*
Control Group (p(FDR) < 0.05)						
Temporal lobe						
Right superior temporal gyrus	41	51.06	-21.86	6.90	3277	6.87
Left superior temporal gyrus	41	-49.52	-25.51	9.03	2575	7.05
Left Inferior temporal gyrus		-45.11	-70.94	1.52	499	6.48
Right middle temporal gyrus	37	44.32	-63.89	1.36	205	6.25
Occipital Lobe						
Right middle occipital gyrus		33.82	-69.26	9.47	193	6.03
HIV Group (p(FDR) < 0.05)						
Temporal lobe						
Right superior temporal gyrus	41	46.36	-19.49	6.18	1248	7.38
Left superior temporal gyrus	41	-51.48	-25.67	6.06	1936	7.27

[a]Co-ordinates are Talairach co-ordinates of the peak voxel

[b]Voxel size refers to the 1.0 x 1.0 x 1.0 mm resolution of the iso-voxeled structural images

The results and regions in Table 29 are described in Figure 24.

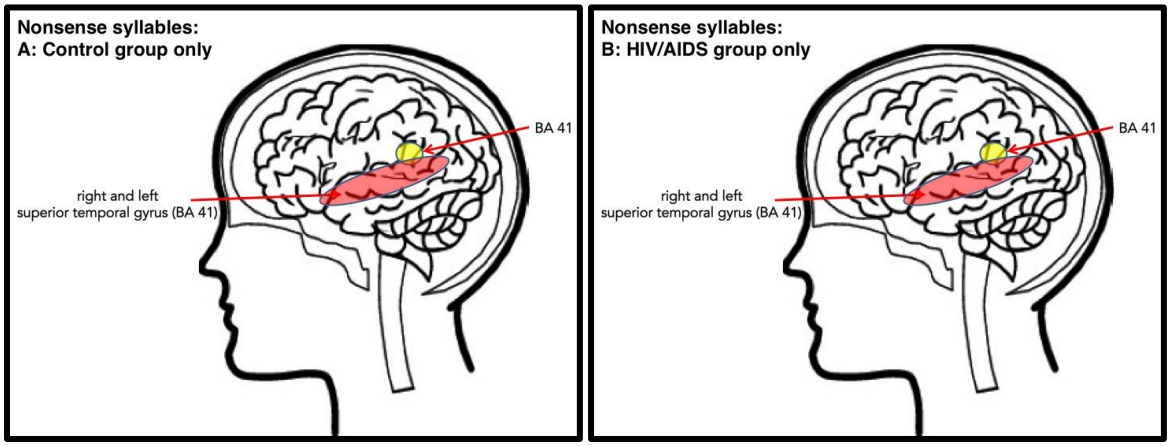


Figure 24: The regions showing significantly more activity compared to baseline in A: control group and B: HIV/AIDS group

The participants in the control group (A) showed significantly ($p < 0.05$ whole brain corrected) *increased brain activation compared to baseline* in the *temporal lobe*: right superior temporal gyrus [51.06, -21.86, 6.90] BA41, total of 3277 voxels and left superior temporal gyrus [-49.52, -25.51, 9.03] BA41 total of 2575 voxels.

The participants in the HIV/AIDS group (B) also showed significantly ($p < 0.05$ whole brain corrected) *increased brain activation compared to baseline* in the *temporal lobe*: right superior temporal gyrus [46.36, -19.49, 6.18] BA41 total of 1248 voxels and left superior temporal gyrus [-51.48, -25.67, 6.06] BA41 total of 1936 voxels.

The patterns, however, differ. To illustrate the activation patterns that occurred in the whole brain for these two groups, threshold weighted overlap maps were used. These maps are functional overlap maps that provide a quick and easy image of the whole brain response to the nonsense syllables sounds and indicates where the activations were at that given voxel. This method is a useful tool to assess the brain visually. These threshold weighted overlap maps (Figure 25) are presented in three orthogonal views (sagittal, axial, and coronal). These views show the bilateral auditory fMRI activation of the auditory cortex areas of both groups in response to auditory stimuli in the nonsense syllables task. The regions were defined as clusters of voxels that were responsive when listening to nonsense syllables.

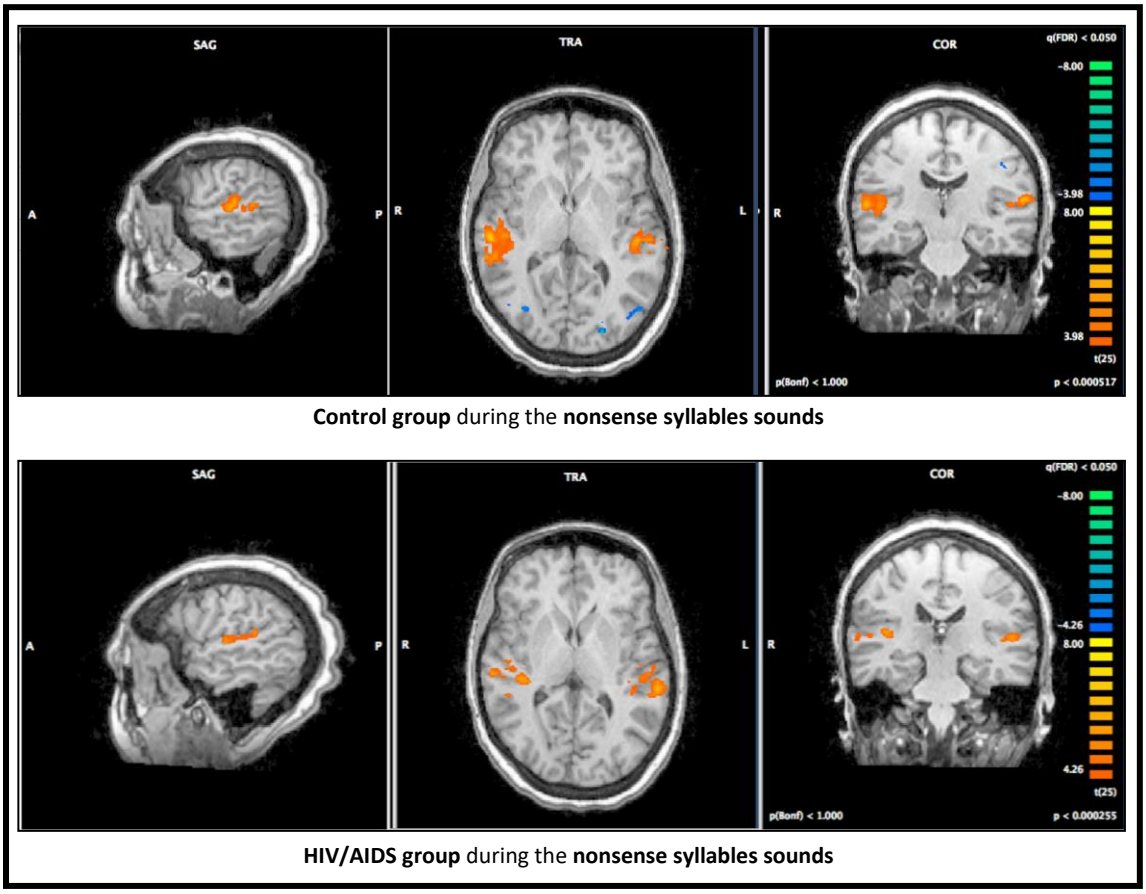


Figure 25: Threshold weighted overlap maps in the control and HIV/AIDS group

The difference in threshold weighted overlap maps between the two groups (control and HIV/AIDS) is clearly pointed out in these activation maps.

Table 30 and Figure 26 described the regions that show *significantly increased brain activation* ($p<0.05$) during the nonsense syllables task in the one group, compared to the other group. The voxel by voxel comparison of the activated areas with voxels above 300 will be used and noted.

Table 30: Regions showing group differences (p(FDR) <0.05) in activation during the nonsense syllables task (voxelwise threshold of 50 contiguous voxels).

Region	Brodmann area	Mean x^a	Mean y^a	Mean z^a	Number of voxels[b]	Max t
Control Group > HIV Group (p(FDR) < 0.05)						
Frontal lobe						
Right middle frontal gyrus		40.3	29.59	23.89	727	4.69
Left inferior frontal gyrus		-49.3	20.91	2.26	294	3.27
Anterior lobe						
Right culmen		4.37	-47.85	-21.85	230	3.60
Parietal lobe						
Left sub-gyral		-32.48	-49.83	39.14	460	4.23
Left postcentral gyrus	40	-61.03	-21.67	14.34	478	3.83
Temporal lobe						
Left superior temporal gyrus	41	-46.17	-31.63	12.6	277	3.09
HIV Group > Control Group (p(FDR) < 0.05)						
Frontal lobe						
Right sub-gyral		16.85	26.66	41.26	214	3.13
Left medial frontal gyrus		-7.46	58.66	15.59	275	2.87
Left sub-gyral		-16.11	22.93	42.32	271	3.77
Left superior frontal gyrus		-18.68	36.31	38.82	318	3.52
Parietal lobe						
Right precuneus	7	11.45	-52.8	49.19	283	3.46
Temporal lobe						
Right sub-gyral		34.2	-70.66	13.87	220	3.84
Occipital lobe						
Right middle occipital gyrus		37.42	-80.6	3.30	281	2.90
Sub-lobar						
Right extra-nuclear		34.14	8.61	-9.09	306	3.50
Right lentiform nucleus; putamen		22.84	8.42	-0.52	291	3.59
Right extra-nuclear; corpus callosum		2.79	25.12	2.66	496	3.90
Left sub-lobar extra-nuclear		-14.22	-43.41	24.85	257	3.46
Left sub-lobar extra-nuclear		-30.36	-42.9	10.6	494	4.05
Left insula		-37.92	9.98	-6.21	510	4.13
Limbic lobe						
Right parahippocampal gyrus	19	26.54	-49.74	-3.35	219	3.06
Right anterior cingulate		13.14	33	22.11	234	4.13

[a]Co-ordinates are Talairach co-ordinates of the peak voxel

[b]Voxel size refers to the 1.0 x 1.0 x 1.0 mm^3 resolution of the iso-voxeled structural images

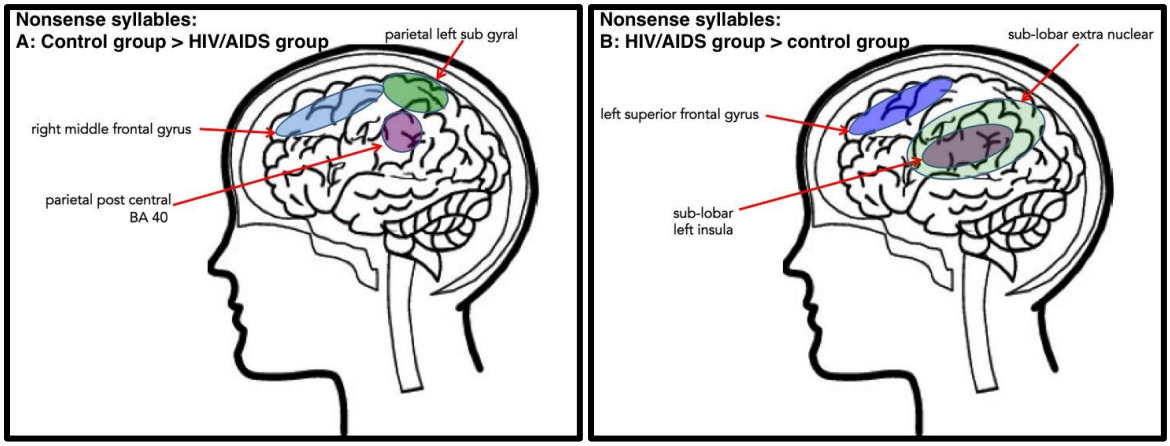


Figure 26: A: control group greater activation than HIV/AIDS group; B: HIV/AIDS group greater activation than control group.

The control group, when compared to the HIV/AIDS group (A), showed a *greater activation* ($p<0.05$) in the following parts of the *frontal lobe:* right middle frontal gyrus [40.3, 29.59, 23.89] total of 727 voxels; *parietal lobe:* left postcentral gyrus [-61.03, -21.67, 14.34] BA 40 total of 478 voxels; left sub–gyral [-32.48, -49.83, 39.14] total of 460 voxels.

The HIV/AIDS group, when compared to the control group (B), showed a *greater activation* in the following parts of the *frontal lobe:* left superior frontal gyrus [-18.68, 36.31, 38.82] total of 318 voxels; *Sub-lobar*: right extra nuclear [34.14, 8.61, -9.09] total of 306 voxels, right extra nuclear corpus callosum [2.79, 25.12, 2.66] total of 496 voxels, left extra nuclear[-30.36, -42.90, 10.60] total of 494 voxels left insula[-37.92, 9.98, -6.21] total of 510 voxels.

Whole brain analysis of the response to warble tones

In the whole brain analysis of the response to warble tones it was clear that the activation patterns were less extensive than for the nonsense syllables. Thus, the active regions were reported for the warble tones if their voxelwise threshold extent was greater than 150 contiguous voxels with a FDR corrected $p < 0.05$, where voxel size refers to the 1.0 x 1.0 x 1.0 mm resolution of the iso-voxeled structural images (Hall et al., 1999).

In Table 31 and Figure 27 the regions that show *greater activation* ($p < 0.05$) during the warble tone task, compared to *baseline*, are summarised for the control group and HIV/AIDS group respectively. The voxel by voxel comparison of the activated areas with voxels above 1 000 will be used and noted.

Table 31: Regions showing significantly greater activity during the warble tone task compared to baseline (voxelwise threshold of 150 contiguous voxels)

Region	Brodmann area	Mean x[a]	Mean y[a]	Mean z[a]	Number of voxels[b]	Max t
Control Group (p(FDR) < 0.05)						
Frontal lobe						
Right Sub-Gyral		32.64	3.56	31.28	360	6.12
Left Sub-Gyral		-32.43	-36.89	29.03	197	5.59
Left Sub-Gyral		-36.36	7.61	24.48	263	5.36
Left Precentral Gyrus		-53.35	3.75	12.83	182	5.28
Anterior lobe						
Left Culmen		-2.98	-52.38	-1.22	251	6.06
Left Culmen		-24.47	-44.05	-20.93	150	5.47
Parietal lobe						
Right Sub-Gyral		33.02	-45.66	24.72	684	5.82
Right Precuneus		17.61	-55.13	42.35	228	5.21
Right Precuneus	7	-13.11	-55.87	50.45	451	5.56
Temporal lobe						
Right Superior Temporal Gyrus		57.37	-13.53	9.87	312	5.33
Right Sub-Gyral		31.57	-63.9	23.9	393	5.51
Left Sub-Gyral		-41.93	-45.97	-8.76	307	5.43
Occipital lobe						
Right Lingual Gyrus		20.88	-77.05	5.28	274	5.23
Left Cuneus		-12.4	-70.04	10.46	206	5.25
Sub-lobar						
Right Extra-Nuclear		23.77	-7.34	18.74	218	5.08
Right Thalamus		13.05	-11.83	7.4	283	5.07
Right Lateral Ventricle		11.2	-22.29	20.48	250	5.43
Left Extra-Nuclear		-5.46	-3.88	11.6	151	5.72
Left Lateral Ventricle		-13.34	-31.54	17.21	700	5.41
Left Lateral Ventricle		-14.56	11.1	21.99	163	5.88
Left Extra-Nuclear		-22.25	-5.04	22.51	190	5.01
Left Extra-Nuclear		-32.9	-1.67	-0.24	1026	5.68
Limbic lobe						
Right Sub-Gyral		35.49	-49.19	-4.8	506	5.20
Right Parahippocampal Gyrus		30.48	-6.32	-11.06	484	5.59
Right Parahippocampal Gyrus		15.59	-39.74	-1.32	314	6.80
Left Posterior Cingulate		-9.39	-47.28	13.34	366	5.42
Anterior lobe						
Left Culmen		-2.98	-52.38	-1.22	251	6.06
Left Culmen		-24.47	-44.05	-20.93	150	5.47
Posterior lobe						
Right Declive		28.77	-53.14	-14.39	181	5.28
Right Pyramis		25.9	-75.73	-29.17	173	5.19
Right Cerebellar Tonsil		16.29	-48.22	-40.42	160	5.08
Left Pyramis		-26.84	-76.48	-29.18	224	5.03
Left Declive		-27.56	-57.68	-18.66	198	5.72

Region	Brodmann area	Mean x[a]	Mean y[a]	Mean z[a]	Number of voxels[b]	Max t
HIV Group (p(FDR) < 0.05)						
Frontal lobe						
Left Sub-Gyral		35	-31	-20	212	3.50
Temporal lobe						
Right Superior Temporal Gyrus		53	7	-6	238	3.56
Right Sub-Gyral		41	-47	24	943	3.31
Left Sub-Gyral		32	-62	24	411	3.67
Left Middle Temporal Gyrus	37	-46	-59	0	341	3.30
Left Inferior Temporal Gyrus	20	-49	-17	33	4638	3.14
Left Inferior Temporal Gyrus		-55	-20	-15	308	2.83
Sub-lobar						
Right Extra-Nuclear		35	-29	5	2767	4.02
Left Extra-Nuclear		-16	-41	24	1581	3.63
Limbic lobe						
Left Posterior Cingulate		-22	-59	6	156	2.69
Posterior lobe						
Right Cerebellar Tonsil		20	-46	-42	237	2.88
Left Declive		-37	-80	-18	2471	3.52

[a]Co-ordinates are Talairach co-ordinates of the peak voxel

[b]Voxel size refers to the 1.0 x 1.0 x 1.0 mm^3 resolution of the iso-voxeled structural images

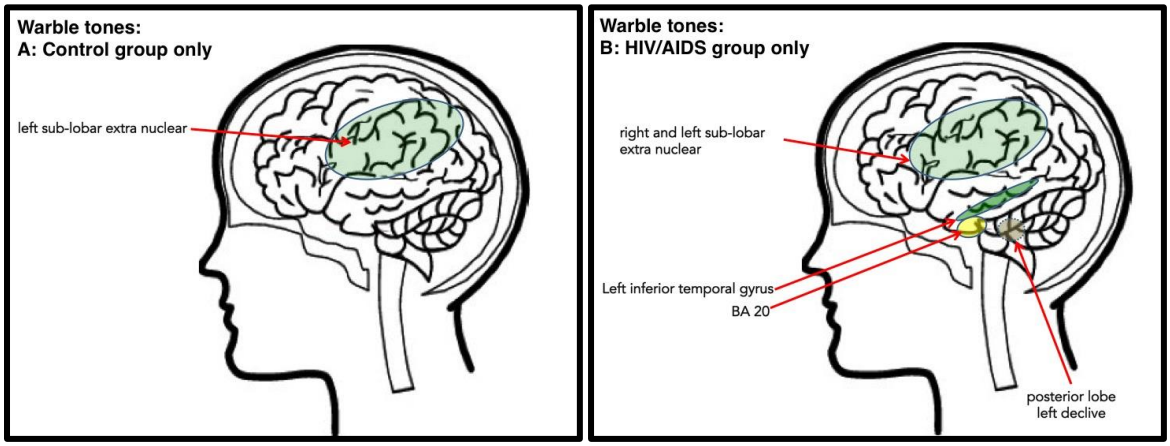


Figure 27: The regions showing greater activity compared to baseline in A: control group and B: HIV/AIDS group

For the purpose of Table 31 and Figure 27 the voxel by voxel comparisons were conducted with a cluster of 1 000 voxels and more.

During the warble tones task compared to baseline with a voxelwise threshold of 150, using only clusters of voxels above 1 000, the control group (A) showed *greater activation* ($p<0.05$) in the whole brain (corrected) compared to baseline, in the sub-lobar part: left extra nuclear [-32.9, -1.67, -0241] total of 1 026 voxels.

During the warble tones task, the HIV/AIDS group (B) showed *greater activation* ($p<0.05$) compared to baseline, in the temporal lobe: left inferior temporal gyrus [-49, -17, 33] BA 20 total of 4 638 voxels; in the posterior lobe: left declive [-37,-80,-18] total of 2 471 voxels; in the sub-lobar part: right extra nuclear [35,-29,5] total of 2767 voxels, sub-lobar left extra nuclear [-16,-41,24] total of 1581 voxels, and the posterior left declive [-37, -80, -18] total of 2 471 voxels.

To illustrate the activation patterns that occurred in the whole brain for these two groups, threshold weighted overlap maps were used once again. These threshold weighted overlap maps (Figure 28) are presented in three orthogonal views (sagittal, axial, and coronal) to show the bilateral auditory fMRI activation of the auditory cortex areas of both groups in response to auditory stimuli in the nonsense syllables task. The regions were defined as clusters of voxels that were responsive while listening to warble tones.

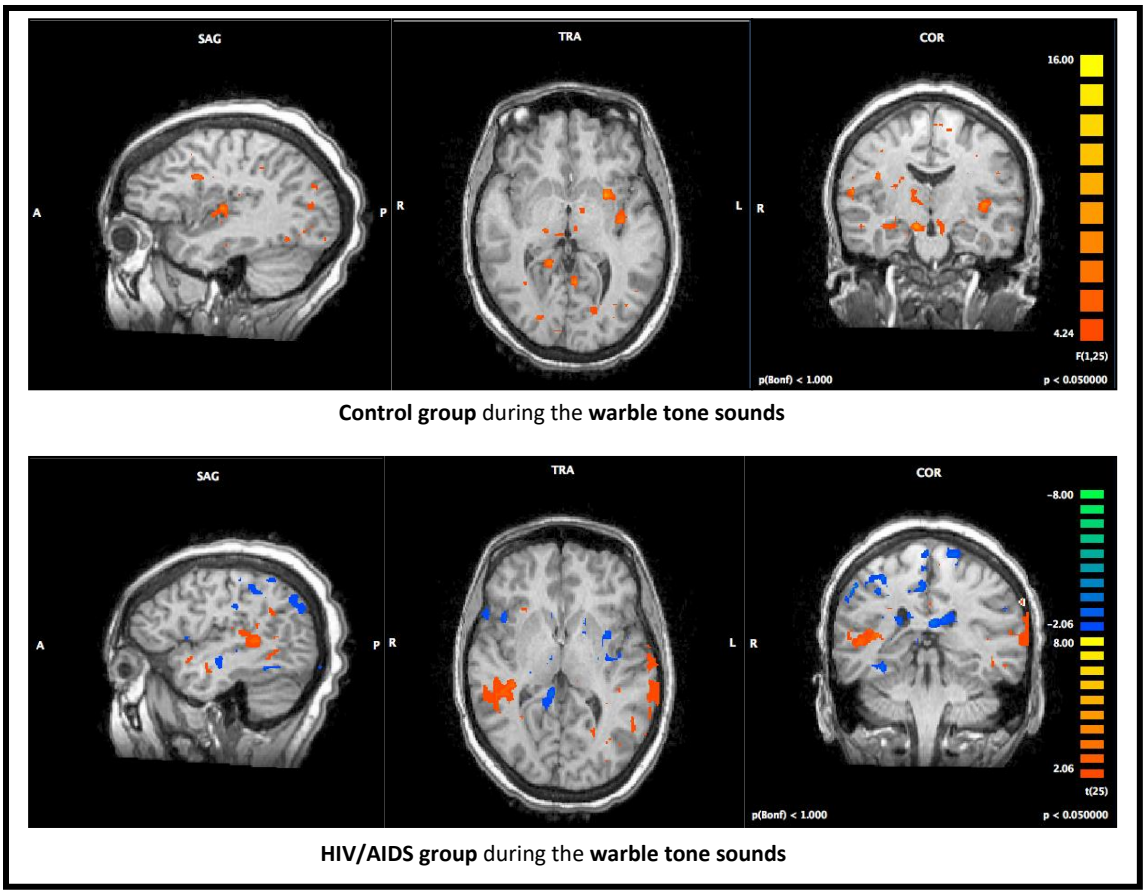


Figure 28: Activation maps in the control and HIV/AIDS group

The difference in threshold weighted overlap maps between the two groups (control and HIV/AIDS) is clearly pointed out in these activation maps.

Table 32 and Figure 29 described the regions that show *greater activation* ($p < 0.05$) during the nonsense syllables task in the one group, compared to the other group. The voxel by voxel comparison of the activated areas with voxels above 300 will be used and noted.

Table 32: Regions showing group differences (p(FDR) <0.05) in activation during the warble tone task (voxelwise threshold of 150 contiguous voxels)

Region	Brodmann area	Mean x^a	Mean y^a	Mean z^a	Number of voxels[b]	Max t
Control Group > HIV Group (p(FDR) < 0.05)						
Frontal lobe						
Right Inferior Frontal Gyrus		35	7	32	270	2.43
Anterior lobe						
Left culmen		29	-53	-15	152	2.28
Parietal lobe						
Left Precuneus		-13	-53	51	379	2.33
Temporal lobe						
Right Precentral Gyrus		56	-11	12	231	2.29
Sub-lobar						
Right Lentiform Nucleus		26	-8	-6	327	2.36
Right Thalamus		14	-14	6	215	2.24
Left Lateral Ventricle		-13	-26	21	561	2.30
Left Lentiform Nucleus		-25	7	0	790	2.36
Limbic lobe						
Right Parahippocampal Gyrus	30	17	-41	-3	255	2.56
Posterior lobe						
Left Pyramis		-25	-74	-30	157	2.24
HIV Group > Control Group (p(FDR) < 0.05)						
Frontal lobe						
Left sub-gyral		-37	10	24	179	2.31
Left Precentral Gyrus		-52	4	12	161	2.27
Parietal lobe						
Right Sub-Gyral		32	-50	27	567	2.38
Right Precuneus		14	-62	30	176	2.27
Left Sub-Gyral		-31	-38	30	152	2.33
Temporal lobe						
Right sub-gyral		32	-62	24	299	2.34
Occipital lobe						
Right Fusiform Gyrus	37	32	-47	-9	419	2.26
Right Cuneus		20	-77	12	221	2.27
Sub-lobar						
Right extra-nuclear		20	-8	21	156	2.26
Limbic Lobe						
Left Posterior Cingulate		-10	-50	15	289	2.30

[a]Co-ordinates are Talairach co-ordinates of the peak voxel

[b]Voxel size refers to the 1.0 x 1.0 x 1.0 mm^3 resolution of the iso-voxeled structural images

Table 32 lists the regions showing *greater activation* during the warble tones task (with a significantly FDR or false discovery rate threshold of $p < 0.05$). The regions and results in Table 32 are described graphically in Figure 29.

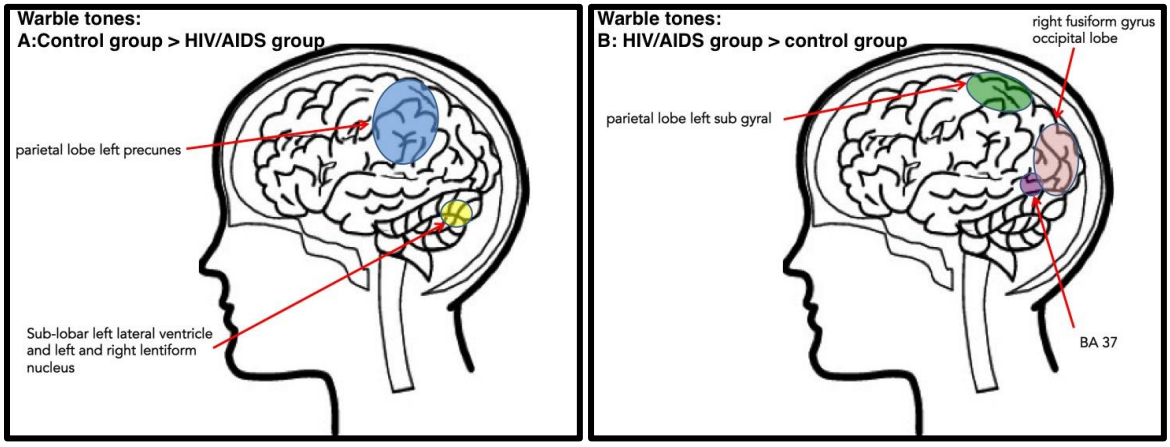


Figure 29: A: control group greater activation than HIV/AIDS group
B: HIV/AIDS group greater activation than control group

For the purpose of Table 32 and Figure 29 the voxel by voxel comparisons were conducted with a cluster of 1 000 voxels and more.

During the warble tones task the control group, when compared to the HIV/AIDS group, showed *greater activation* in the *parietal lobe:* left precuneus [-13,-53,51] total of 379 voxels, in the *sub-lobar:* right lentiform nucleus [26,-8,-6] total of 327 voxels, lateral ventricle [-13, -26, 21] total of 561 voxels, left lentiform nucleus [-25, 7, 0] total of 790 voxels.

The HIV/AIDS group, when compared to the control group, showed a *greater activation in parietal lobe:* right sub-gyral [32, -50, 27] total of 567 voxels, *occipital lobe:* right fusiform gyrus BA 37 [32, -47, -9] total of 419 voxels.

4.5. Chapter summary

This chapter provided an overview of the results obtained in this exploratory study. The presentation included quantitative data with inferential statistics according to the sub-aims specified for this study. Taken together, the results of the ROIs and whole brain analyses show different brain activation in the different participant groups. Voxel by voxel comparisons in the brain were implemented. These results will be discussed in Chapter 5.

CHAPTER 5
DISCUSSION OF RESULTS

"Discussion is an exchange of knowledge"
(Robert Quillen)

5.1. Introduction

In the previous chapter, the results were presented corresponding to the sub-aims of this study. The intention of Chapter 5 is to discuss the results obtained in this study and to explain the meaning, significance, and implications of the findings. This will be done in accordance with existing literature in this field of study, by critically comparing research methodologies and findings and attempting to draw conclusions regarding the response of the CANS to sound in normal hearing adults with and without HIV/AIDS.

5.2. Discussion sub-aim one

Sub-aim one: To determine the response of the CANS (ROI) to nonsense syllables in normal hearing participants with and without HIV/AIDS, using fMRI.

Firstly, the results as reported in Chapter 4 showed a significant difference between the two participant groups in the response of the CANS to nonsense syllables.

Secondly, the results were controlled for confounding influences such as gender, age, and education on the significant mean percentage signal change of level of activation in BA41 and BA42, by employing statistics to compare the results from the two groups according to gender, age, and education.

Thirdly, it was also considered relevant to determine whether the CD4 count or ART had any effect on the neural response of the CANS (ROIs) to auditory stimulation in the HIV/AIDS group.

These three aspects are discussed separately:

5.3. The response of the CANS (ROIs) to nonsense syllables

When the two groups of participants were compared, there was a significant difference of activation for nonsense syllables observed in both BA41 ($p = 0.03$) and BA42 ($p = 0.03$) ROI's, in the right and the left hemispheres of the brain. The control group showed a higher mean percentage signal change in BA41 ($M = 0.154$) and in BA42 ($M = 0.154$), while the HIV/AIDS group showed a lower mean percentage signal change ($M = 0.107$) in BA42 ($M = 0.068$). The control group showed a significantly greater activation bilaterally than the HIV/AIDS group in both these areas although both groups had normal hearing.

The findings with regard to greater activation in the control group in the current study may bear relation to the results reported by Koshino, Carpenter, Minshew, Cherkassky, Keller and Just (2005), whose findings are consistent with the "functional underconnectivity" theory of autism. In their study, the experimental group showed lower synchronization among the brain areas than the control group in general. The outcome is manifested as deficient higher order cognitive processes, reduced fMRI activation of regions performing integrative processing during complex tasks, and reduced synchronisation and a local processing approach to cognitive challenges (Just, Cherkassky, Keller & Minshew, 2004). The results of the present study suggest that the degree of underconnectivity in participants with HIV/AIDS depended on the task requirements and brain regions recruited for the task.

The PAC includes the areas of BA41 and BA42. In a study by Bernal and Ardila (2016), the PAC showed sensitivity to speech sounds and was proven to be involved in phonological processing. In accordance with the findings of the current study, BA41 and BA42 activation was more significant when nonsense syllables were presented. Bernal and Ardila (2016) include BA41 and BA42 as part of the perceptual language area and confirm that the left hemisphere typically exerts an inhibitory tone over the right, so that language is left-lateralised

(as was the case for the participants of their study). Binaural interaction with sine tones for auditory processing is a simple method of activating Heschl's gyrus and the planum temporale (Di Salle, Esposito, Scarabino, Formisano, Marciano, Saulino, Cirillo, Elefante, Scheffler and Seifritz, 2003). Thus, while both hemispheres were involved in the analysis of all the nonsense syllables responses of this study, involvement was stronger in the BA41 and BA42 areas, both of which are part of the PAC.

The function referred to as phonological processing (phoneme recognition) is found in the BA41 and to a lesser extent in the BA42 region. Heschl's gyrus is the intersection area where auditory processing (auditory stimulus) and language processing (meaning) occur (Richard, 2001). Both BA41 and BA42 have perceptual functions and are part of the main language processing area also known as Wernicke's area. Wernicke's area includes BA21, BA22, BA41, and BA42. These areas are partly responsible for language processing and recognition (Ardila et al., 2016). Masters and Ances (2014) observed that HIV/AIDS participants showed thinner cortical thickness, smaller cortical volumes, and a larger ventricular size than control participants. This could be related to the degree of neurocognitive impairment. fMRI studies have the potential to be valuable for observing or detecting functional changes in specific areas, such as BA41 and BA42 as was found in the current study.

Neuroimaging techniques such as fMRI produce maps of cortical activation rely on changes in hemodynamic variables occurring in close proximity to localized increases of neural activity (Harris, Jones, Zheng & Berwick, 2010). According to Masters and Ances (2014), fluctuations in the BOLD response within specific brain regions reveal a decrease or increase in neuronal activity as determined by cerebral blood flow to those specific areas under investigation. A decrease in activation, which is caused by less oxygenated blood flow to the regions, might thus be an indication of less activity in this area. In a study by Pluta et al. (2011), the results indicated smaller or less activation within specific brain areas in participants with CAPD, where the display was smaller or less activation was observed within certain areas of the brain. The difference in brain activity noticed in participants in this current research study might be dissimilar to the decreased activation described in the account of behavioural problems in participants with CAPD and HIV/AIDS and the control participants.

Kallail, Downs, Scherz, Sweet and Zackula (2014) found that participants with HIV/AIDS manifested cognitive impairments related to the CNS as well as communication deficits even if they were fairly healthy. Kallail et al. (2014) found a high prevalence of cognitive and language deficits and central auditory disturbances in persons with HIV/AIDS. The cognitive impairments found in their HIV/AIDS participants had an effect on speech, language, and hearing, which influenced their communication abilities. Additional research on how the communication ability of people with HIV/AIDS is affected is necessary to maintain and improve their communication skills and quality of life (Kallail et al., 2014).

HIV associated neurocognitive disorder (HAND) is a collective term for neurocognitive disorders caused by HIV/AIDS (Nabha, Duong & Timpone, 2013). HAND starts sub-clinically before it progresses to a symptomatic stage (the primary disease or disorder) (Hakkers et al., 2017). BOLD fMRI has been proved to be a sensitive tool to detect abnormal brain function in an early stage and might therefore be a useful tool to evaluate the effect of HAND on the brain function (Hakkers et al., 2017). It has been shown that HAND can cause low concentration, memory loss, mental slowness, and decrease in motor symptoms (Nabha et al., 2013). A comprehensive neurocognitive evaluation for evaluation of HAND should therefore involve the five neurocognitive areas: verbal/language, attention/working memory, abstraction/executive function, learning/recall, rate of information processing, and motor skills.

The significant difference in activation in the ROIs found in this study in response to the presentation of nonsense syllables should be investigated further to determine if activation changes can be seen as underlying the differences in functioning found in the research discussed above.

Possible confounding factors that may have an influence on the differences in activation observed in BA41 and BA42

To further investigate the response of the CANS (ROIs) to nonsense syllables, the signal changes in the area of BA41 and BA42 were statistically analysed to determine if gender, age, and education affected the results for all the participants.

- **Age and education**

The influence of possible confounding factors such as age and education on the mean percentage signal change in BA41 and BA42 in the two groups was also determined. The results showed that age BA41 ($p = 0.95$) and BA42 ($p = 0.85$) and education BA41 ($p = 0.68$) and BA42 ($p = 0.99$) had no statistically significant effect on the mean percentage signal change in BA41 and BA42 for any of the two participant groups.

- **Gender**

The influence of possible confounding factors such as gender on the mean percentage signal change in BA41 and BA42 in the two groups was also determined. The results showed that gender had a statistically significant effect on the mean percentage signal change in BA41 ($p = 0.042$) and BA42 ($p = 0.037$) for the two groups in this study. The interaction between gender (male and female) in the two groups (HIV/AIDS and control) showed a difference.

With regard to the independent variables gender and group, there was no significant difference between the male participants of the two groups ($p = 0.614$), but a significant difference was found between female participants ($p = 0.008$), with the mean percentage signal change (M) in the HIV/AIDS group reported as $M = 0.088$ and in the control group as $M = 0.170$ for the areas BA41 and BA42.

A study by Harasty, Double, Halliday, Kril and McRitchie (1997) revealed that the language-associated cortical regions (Wernicke and Broca) in the female brain are proportionally larger than those of males. The volume of the superior temporal cortex, the planum temporale as well as the cortical volume fraction of the Broca area, were significantly larger in females when compared to those of males. The anatomical differences may correlate with the results in the current study, where the control group females had better mean percentage signal change in BA41 and BA42 than the male participants. Gender differences have been detected with fMRI for cognitive and language processing. Language processing is considered a gendered variable. Females appear to have an advantage over men in language production. fMRI studies employing a variety of data processing procedures (Kaiser, Haller, Schmitz & Nitsch, (2009) have confirmed gender related differences in language production.

In addition, an fMRI study done by Burman, Bitan and Booth (2008) showed stronger activation of the cortical language processing areas in females compared to males (Richardson, Martin, Jimenez, Danley, Cohen, Carson, Sinclair, Racenstein, Reed and Levein, 2001).

Maki and Martin-Thormeyer (2009) found a significantly higher prevalence of neurocognitive impairment among women with HIV/AIDS compared to women without HIV/AIDS. In the current study the HIV/AIDS males had better mean percentage signal change in BA41 and BA42 than the HIV/AIDS females. This should be considered in conjunction with the report that women with HIV/AIDS are at increased risk for neuropsychological impairment compared to women without HIV/AIDS. The proportion of women affected by HIV/AIDS varies by global region. In sub-Saharan Africa, nearly 60% of those infected with HIV/AIDS are women. Additional studies implementing neuroimaging could focus on the factors that influence cognitive dysfunction in HIV/AIDS, and correlations between cognitive impairments such as HAND in women living with HIV/AIDS.

Younger people are the most severely affected by HIV/AIDS. It is estimated that over 60% of all new infections currently occur among individuals between 15 and 25 years of age, with women generally being infected earlier than men (Life, 2000). Data from a study by Gilgen, Campbell, Williams, Taljaard and Macphail (2000) support and augment this worrying situation. In a mining town in Gauteng an extraordinarily high rate of infection was found among adolescent girls, reaching nearly 60% at the age of 25. Overall, this provides a general picture of the patterns in gender disparity in HIV/AIDS infection across countries in sub-Saharan Africa, that more women are affected by HIV/AIDS than men, and that more women with HIV/AIDS are affected by other diseases than women without HIV/AIDS.

There is a pressing need for more large-scale studies of neuropsychological function in women with HIV/AIDS. Such studies are needed to address neurocognitive complications of HIV/AIDS in women, to identify factors that contribute to these complications, and to develop possible treatments for some of the factors contributing to the cognitive dysfunction (Maki et al., 2009).

5.4. The effect of CD4 count and ART on the activation (mean percentage signal change) in BA41 and BA42

As noted, the results of the current study disclosed that HIV/AIDS disease severity in terms of the CD4 count and the activated BOLD signals in BA41 and BA42 area during the nonsense syllables task showed a tendency in terms of the higher the CD4 count in the HIV/AIDS group, and the lower the activation of the signal change in BA41 or BA42.

An inverse correlation was found between the years on ART and the activated BOLD signals in BA41 and BA42 areas during the nonsense syllables task. The random effects linear regression model in SPSS showed inverse correlations between the mean percentage signal change in either BA41 or BA42, and ART use in years. As the period of use of ART (in years) increases, the mean percentage signal change in BA41 or BA42 decreases. This negative response to the mean percentage signal change reflects a decrease in oxygen consumption and neural activity, which can be induced by a reallocation of blood flow from the less demanding areas to the most demanding regions in the brain.

Although the results revealed that HIV/AIDS disease severity in terms of the CD4 count and the activated BOLD signals in BA41 and BA42 area during the nonsense syllables task showed a tendency (the higher the CD4 count in the HIV/AIDS group, the lower the activation of the signal change in BA41 or BA42), it would require a bigger sample to shed more light on these findings.

Melrose, Tinaz, Castelo, Courtney and Stern (2008) found that HIV/AIDS participants using ART demonstrated an inverse correlation between CD4 count and functional BOLD signal. According to Melrose and colleagues (2008), the use of ART had no effect on the BOLD signal of fMRI examination.

In a study involving HIV/AIDS positive patients, Küper, Rabe, Esser, Gizewski, Husstedt, Maschke and Obermann (2011) discovered that prefrontal gray matter atrophy in the HIV/AIDS patients was associated with the disease duration. The lengthier the disease, the more it had an effect on motor dysfunction, and this was related to basal ganglia gray matter atrophy. There is a correlation between the CD4 cells count and the occipital gray matter. A

decrease of occipital gray matter will follow if the CD4 cells count decreases. There is evidence of atrophy of nigro-striatal and fronto-striatal circuits in HIV/AIDS patients (Kuper et al., 2011).

Significant differences in brain activation have been found between untreated HIV/AIDS persons and HIV/AIDS persons on ART treatment (Chang, Yakupov, Nakama, Stokes & Ernst, 2008). It was found that the higher the CD4 count in the untreated HIV/AIDS group, the lower the activation of signal change in BA41 and BA42. The CD4 count appears to be related to the functioning of BA41 and BA42. In the HIV/AIDS group on ART treatment it was found that the longer they had been using ART, the less percentage signal change there was in BA41 and BA42.

These results correlate with a study by Mrudula, Suwarna, Khadse, Minal and Shubhangi (2012), who observed that after receiving ART, the mean CD4 count of patients with HIV/AIDS decreased from 336 to 226. It was proposed that the patients who showed a rise and those who showed a fall in CD4 count after ART should be evaluated separately. Nearly 75% of adults with HIV/AIDS present some type of hearing impairment due to opportunistic infections or treatments with ototoxic medications suggesting impairment in the peripheral auditory pathway. The group exposed to ART presents higher proportion of alterations than the group not exposed to ART treatment (Matas et al., 2014). ART treatments are effective and people on treatment do live longer, but cognitive impairment in a milder form has been noted in these individuals. This can be due to the longer duration of living with HIV/AIDS while on ART. ART can also affect sensory functions with a resultant impact on various aspects of cognitive functioning. Current evidence supports the concept that HIV/AIDS has significant manifestations that relate to hearing loss due to the direct effects of the HIV/AIDS on the CANS, opportunistic infections, and ototoxicity through the treatment of opportunistic infections and administering of ART (Stearn & Swanepoel, 2010; Khoza & Ross, 2002; Chandrasekar et al., 2000; and Khoza-Shangase, 2010).

Liner, Ro and Robertson (2010) discussed various studies, including studies in Africa that investigated ART effects on neurocognitive impairment. Progress has been made to reveal evidence of neurocognitive impairment in people living with HIV/AIDS in South Africa and Zambia, with deficits generally observed in areas of executive functions, information processing, episodic memory, working memory, and language.

In a report by Klunder, Chiang, Dutton, Lee, Toga, Lopez, Aizenstein, Becker and Thompson (2008), it was mentioned that ART has greatly increased life expectancy for many of those infected with HIV/AIDS, but as patients survive longer, there is increasing concern that chronic viral neurotoxicity can lead to progressive brain atrophy and associated neurocognitive impairment in some participants (Torre et al., 2009). There seems to be consensus that ART exposure may be a risk factor for hearing loss (Torre et al., 2015).

Hakkers et al. (2017) state that fMRI can be a sensitive instrument to detect subtle cognitive changes in HIV/AIDS participants and shed light on the effects of ART and the effects of aging.

5.5. Discussion sub-aim two

Sub-aim two: To determine the response of the CANS (ROI) to warble tones in normal hearing participants with and without HIV/AIDS, using fMRI.

The results indicated a marginally significant difference between the HIV/AIDS group and the control group with regard to the response of the CANS (ROI) to warble tones. This was especially evident in the right inferior colliculus (IC) ($p = 0.07$). The HIV/AIDS group had a lesser activation than the control group in this area. Stated otherwise, more activation was observed in the right inferior colliculus of the control group than in the HIV/AIDS group. The control group showed more mean percentage signal change in the right inferior colliculus ($M = -0.002$), while the HIV/AIDS group showed a lesser mean percentage signal change in the right inferior colliculus ($M = -0.046$). These actual negative BOLD responses were associated with a decrease in oxygen consumption and neural activity. These results also suggested that meaningful, stimulus-specific processing occurs even in regions that display a strong negative BOLD response. Harel, Nagaoka, Kim and Kim (2002) explained that a negative BOLD response reflects a decrease in cerebral blood flow. This can be induced by a reallocation of blood flow from the less demanding areas to the most cerebral blood flow demanding regions in the brain.

In a study by Hawley, Melcher and Fullerton (2005) the IC, SOC, and CN all showed increased fMRI activation with increasing sound bandwidth when noise stimuli were presented. The results provided data on which to base the design of neuroimaging studies that need to be controlled (Hawley et al., 2005). In this study the sound bandwidth was randomly played in five frequencies, the sound consisting of warble tones comprising five frequencies: 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, and 8000 Hz, played at ten seconds per frequency.

Champoux, Paiement, Mercier, Lepore, Lassonde and Gagné (2007) report that the role of the inferior colliculus in human auditory processing is as yet poorly understood. In humans, the principal drawback of the lesion approach, derived from case studies, is that the lesion is rarely so circumscribed as to affect only auditory processing. This is particularly true of the inferior colliculus. To assess the role of the inferior colliculus in human auditory processing Champoux et al. (2007) employed a battery of auditory tests to investigate the influence of a circumscribed lesion of the right inferior colliculus without additional neural damage. The results disclosed bilaterally normal peripheral auditory functioning. The findings confirmed that the inferior colliculus is not involved in peripheral auditory processing. Tonal detection thresholds and frequency-pattern recognition were normal bilaterally. In the study by Hawley et al., (2005), the activation in response to binaural continuous noise of various bandwidths was almost always detected bilaterally in the inferior colliculi (10/10 participants) and cochlear nuclei (9/10 participants). In some participants, activation was also detected in the superior olivary complex (on both sides in 5/10 participants). In the current study there was a marginal difference between the HIV/AIDS group and the control group with regard to the response of the CANS (ROI) to warble tones, and this was especially evident in the right inferior colliculus (IC) ($p = 0.07$).

The result of the current study indicated a marginally significant difference between the HIV/AIDS group and the control group with regard to the response of the CANS (ROI) to warble tones. This may reflect an underlying pathology condition that requires follow-up examination with an audiologist for behavioural audiometric testing and another auditory fMRI. A study by Gu, Halpin, Nam, Levine, and Melcher (2010) involved participants with and without tinnitus, all with clinically normal hearing thresholds, namely pure tone thresholds (25 dB HL) at intervals from 250 Hz to 8000 Hz. The mean pure tone threshold for the tinnitus and non-tinnitus subjects differed by 3 dB or less at any given frequency. Activation was quantified within regions of interest (ROI). These ROIs included three subcortical structures:

the inferior colliculus, medial geniculate body, and cochlear nucleus (Hawley et al., 2005; Harms & Melcher, 2002). The participants with, as well as those without tinnitus showed sound-evoked fMRI activation in the inferior colliculus. A variety of clinical conditions are characterised by disordered perceptions that arise from abnormal elevations in neural activity in the CNS. The results provide strong evidence for a relationship between the magnitude of sound-evoked fMRI activation in the auditory pathway and sound level tolerance, with activation from sound increasing with decreasing tolerance.

In the study by Champoux et al. (2007) it was confirmed that a circumscribed lesion limited to the IC, an upper midbrain structure, does not affect auditory function. The pattern of results suggests that auditory functions such as recognition of low-redundancy speech presented monaurally, recognition of tone duration patterns, binaural separation and integration, as well as sound-source localization, depend on the integrity of the bilateral auditory pathways at the IC level (Champoux et al., 2007).

5.6. Discussion sub-aim 3:

Sub-aim three: To compare the response of the whole brain to nonsense syllables and warble tones in participants with and without HIV/AIDS.

The results of a whole brain analysis did provide noteworthy information about signal activation differences in the individual regions of the brain. When a group of voxels has been identified in the whole brain analysis, an ROI is created to demonstrate whether that cluster is informative, and the properties of that ROI are then characterised. If the ROI made from the cluster is informative, there is justification to conclude that the cluster itself is informative and therefore significant relative to the results in the whole brain (Etzel, Zacks & Braver, 2013). fMRI can provide a brain-based framework for examining the relative levels of activity in specific regions. It can also help to establish whether activation is functionally different in the auditory system of participants with HIV/AIDS. In addition, patterns of activation can reveal differences even in cases where the recorded performance is highly accurate in both controls

and individuals with HIV/AIDS. fMRI offers the possibility, therefore, of examining residual compensatory mechanisms.

This group clustering method was implemented by combining the data to identify the desired number of clusters consisting of voxels. In the current study the activated areas were used for the purpose of determining group differences. It should be noted that the results in this section were analysed by using voxel-by-voxel comparisons (Etzel et.al, 2013).

The nonsense syllables and warble tone tasks can be expected to produce differential patterns of activation across the left and right hemispheres, thus the whole brain analysis was done to determine and compare the multiple areas of activation and responses to nonsense syllables and warble tones.

BOLD imaging is a method used in fMRI to observe different mapping areas of the brain that are found to be active at any given time in response to certain stimuli. During activation of a brain region there is an excess of arterial blood delivered into the area, with concomitant changes in the ratio of deoxyhemoglobin to oxyhemoglobin. The rationale for interpreting these changes as cellular activity is that cells in the brain, like those elsewhere in the body, use oxygen as fuel. As they increase their activity, they increase their demand for oxygen, and the arterial blood vessels respond by delivering more oxygenated hemoglobin (oxyhemoglobin) to the region. For example, when listening to a stimulus (sound or tone), certain areas of the brain require more oxygen and therefore an increased blood flow to those particular areas; consequently, the increased oxygen content in the blood is observed in the BOLD response from the activated areas in the brain. These activated regions consist of clusters that contain voxels that belong to that specific activated region (Harel et al., 2002).

5.6.1. Whole brain analysis of the response to nonsense syllables

In the present study the participants in both the control group and the HIV/AIDS group showed, as expected, increased voxel activation in certain regions of the brain compared to the baseline in response to auditory stimulation. Both groups had increased voxels of activation in the temporal lobe in the right superior temporal gyrus (BA41) as well as in the left superior temporal gyrus (BA41). The control group showed more voxel increased activation than the HIV/AIDS group in the left and right superior temporal gyrus.

Both groups of participants showed increased activation in the temporal lobe when nonsense syllables were presented. The control group showed more voxel activation in the right superior temporal gyrus [51.06, -21.86, 6.90] [BA41, 3277 voxels] and left superior temporal gyrus [-49.52, -25.51, 9.03] [BA41, 2575 voxels] in the temporal lobe. The participants in the HIV/AIDS group showed less voxel activation in the right superior temporal gyrus [46.36, -19.49, 6.18] BA41, 1248 voxels] and left superior temporal gyrus [-51.48, -25.67, 6.06] [BA41 total of 1936 voxels] in the temporal lobe.

The results of a study by Clark, Boutros and Mendez (2010) confirmed that BA41 (consisting of the left and right superior temporal gyrus) of the right and left hemispheres of the brain is part of the auditory association area. The superior temporal gyrus of the left hemisphere of the brain is where speech sounds, rate of speech tempo, and word meanings are processed. Uncomplicated auditory tasks for example passive listening to tones or vowel sounds can activate these areas. The activation can then extend to the anterior and posterior area of the Heschl's gyrus area. Non-meaningful sounds activate the superior temporal sulcus bilaterally (Clark, et al., 2010).

Warrier, Wong, Penhune, Zatorre, Parrish, Abrams and Kraus (2009) confirmed that the left Heschl's gyrus (BA41) structure is implicated in language learning. Warrier et al., (2009) found that participants who successfully learned to associate auditory patterns with word meaning had larger left Heschl's gyrus (BA 41) volume than those who had difficulty learning these associations. Results from Pugh, Shaywitz, Shaywitz, Fulbright, Byrd, Skudlarski, Shankweiler, Katz, Constable, Fletcher and Lacadie (1996) also suggested more left hemisphere activation during speech processing. It must be noted that in the present study a passive listening condition was used.

These findings also concur with the results relating to the first aim of this study where the control group's ROI namely BA41 had a significantly higher mean percentage signal change than in the HIV/AIDS group. Both groups had increased activation in the left superior temporal gyrus (BA41), although the control group had a higher cluster-voxels activation, which reveals activity in these areas in response to auditory stimuli with nonsense syllables. When ROI or whole brain analyses are used with nonsense syllables, the area BA41 is seen to be activated, which implicates the language association area namely the PAC that includes

BA41 and BA42. In these areas, which are important for the processing of speech stimuli, participants with HIV/AIDS showed less activation than the control group did.

HIV/AIDS is associated with central nervous system changes that may affect cerebral blood flow metabolism, structure, and diffusion. This shows that increases/decreases in brain activation during a certain task compared to rest, may be presumed to be related to cognitive function. Research utilizing fMRI in HIV/AIDS suggested that patients with definite cognitive impairment have increased cerebral blood volume compared to people with normal cognitive function. The severity of abnormal activation may depend on task complexity. In comparison to controls, people with HIV/AIDS demonstrated greater parietal activation during a simple task of attention, and greater frontal and parietal activation during a more complex attention task, even prior to the development of clear cognitive deficits. Compared to healthy individuals, people with HIV/AIDS may need to exert extra effort to perform the same task (Tucker, Robertson, Lin, Smith, Chen, Aylward & Hall, 2004).

The control group, when compared to the HIV/AIDS group, showed increased brain activation in the right middle frontal gyrus [40.3, 29.59, 23.89] [727 voxels] in the frontal lobe, and in the left postcentral gyrus [-61.03, -21.67, 14.34] [BA 40, 478 voxels] and left sub-gyral area [-32.48, -49.83, 39.14] [460 voxels] in the parietal lobe. The HIV/AIDS group, when compared to the control group, showed increased brain activation in the left superior frontal gyrus [-18.68, 36.31, 38.82] [318 voxels] of the frontal lobe, and the right extra nuclear area [34.14, 8.61, -9.09] [306 voxels], right extra nuclear corpus callosum [2.79, 25.12, 2.66] [496 voxels], left extra nuclear area [-30.36, -42.90, 10.60] [494 voxels], and left insula [-37.92, 9.98, -6.21] [510 voxels] in the sub-lobar region.

Li, Li, Gao, Yuan and Zhao (2014) established that there are certain main brain regions on which HIV/AIDS can have an effect. These affected areas can cause cognitive impairment that can be part of HAND. The current results confirmed the report by Li et al. (2014) of less activation in the right middle frontal gyrus of the HIV/AIDS group [32, 36, 33]. In the current study the activation in the right middle frontal gyrus [40.3, 29.59, 23.89] is greater in the control group, while there was reduced voxel size in the activation area in the frontal lobe of the HIV/AIDS group. This can suggest that HIV/AIDS participants have reduced attention networking so that the frontal lobes use less oxygen to perform certain tasks compared to the oxygen use of the control group. The control group demonstrated that they were more capable

in performing the listening tasks and they showed more activation in the frontal region, especially the right middle frontal lobe.

On the other hand, the literature does report findings to the contrary. In the study by Chang et al. (2008), HIV/AIDS participants had to perform a task that required attention. Voxel-by-voxel comparisons showed that the HIV/AIDS group had greater load-dependent increase in activation than control subjects in the frontal regions, especially the left superior frontal gyrus [-18, 42, 33][8] [BA 9]. While the control group showed load dependent decreases in BOLD signals in these frontal regions, the HIV/AIDS group showed load dependent increases in these regions. As the brain may have a limited capacity to further increase the BOLD activation response with more difficult tasks, the task performance in the HIV/AIDS participants may decline when more difficult tasks are performed. These findings can correlate with the findings of the current study, although this study used a passive listening task. The left superior frontal gyrus [-18.68, 36.31, 38.82] of the HIV/AIDS group showed more activation than in the case of the control group. Although the tasks were different, both studies suggest greater usage of their brain reserve capacity as the task becomes more difficult.

The control group showed more activation in the left sub-gyral area and the left post central gyrus (BA40), both of which are situated in the parietal lobe of the left hemisphere of the brain. The left post central gyrus (BA40) is also part of Wernicke's area, which is responsible for some complex linguistic processes such as semantic processing and verbal creativity. It is also involved in language perception and processing (Cortical Functions Reference, 2012). In an auditory selective fMRI study by Pugh et al. (1996), the results indicated that conditions that include selective attention activate the parietal lobe in which these areas are situated to a greater extent and it also suggested that left hemisphere activation is greater due to the processing of speech.

In recent human neuroimaging studies, the insula is regarded as an important brain area in the physiological understanding of the brain (Namkung, Kim & Sawa, 2017). Ardila, Bernal and Rosselli (2016) mentioned that the insula (BA13) is part of the language processing complex. Insula connections include areas involved in language processing (Broca's area) and

[8] The coordinates were provided to show how close the coordinates of the other studies are to those of the present study.

language understanding (Wernicke's area) but are also involved in language repetition, linguistic functions, and lexico-semantic associations. The insula (BA13) plays a coordinating role in interconnecting these two brain language systems (lexical-semantic and grammatical). The extranuclear corpus callosum connects the cerebral hemispheres and provides for interhemispheric integration and transfer of information (Fabri & Polonara, 2013). In the current study the HIV/AIDS group had a higher voxel-by-voxel comparison than the control group in the region of the left insula in the sub-lobar part of the brain. As in the study of Ardila et al. (2016) the insula is part of the language processing (Broca's area) and language understanding (Wernicke's area) area.

5.6.2. Whole brain analysis of the response to warble tones

The participants in both the control group and the HIV/AIDS group showed increased brain activation compared to baseline with warble tone stimulation, which is to be expected. The HIV/AIDS group, however, presented with a more scattered cluster-voxels activation, indicating that other association areas were also activated in response to warble tones, in other words when the participants of this group listened to warble tones activation did not always take place in the areas as expected but in conjunction with other associated areas.

In the control group there was increased brain activation in the left extra nuclear area [-32.9, -1.67, -0241] [1 026 voxels] in the sub-lobar region. The participants in the HIV/AIDS group showed increased brain activation in the inferior temporal gyrus [-49, -17, 33] [BA 20, 4 638 voxels] in the temporal lobe, in the left declive [-37, -80, -18] [2 471 voxels] in the posterior lobe, in the right extra nuclear [35, -29, 5] [2 767 voxels] in the sub-lobar region, as well as in the left extra nuclear area [-16, -41, 24] [1 581 voxels] and the posterior left declive [-37, -80, -18] [2 471 voxels] of the sub-lobar region. There is a comparable network of areas of the brain activated in higher-order cognitive processes of the listening tasks, with a high similarity in the cortical areas.

The control group, when compared to the HIV/AIDS group, showed increased brain activation in the left precuneus area [-13, -53, 51] [379 voxels] in the parietal lobe, and in the right lentiform nucleus [26, -8, -6] [327 voxels], lateral ventricle [-13, -26, 21] [561 voxels], and left lentiform nucleus [-25, 7, 0] [790 voxels] in the sub-lobar region. The HIV/AIDS group, when compared to the control group, showed increased brain activation in the in right

sub-gyral area [32, -50, 27] [567 voxels] of the parietal lobe, and the right fusiform gyrus [32, -47, -9] [BA 37, 419 voxels] in the occipital lobe. The two groups differ in activation when listening to warble tones. Activation did not always take place in the areas as expected but in conjunction with other associated areas.

In the HIV/AIDS group a higher voxel count than baseline was found in BA20, also known as the inferior temporal gyrus area [-49, -17, 33] [-55, -20, -15], and part of the temporal cortex. This region is involved in visual processing and also recognition memory (Cortical Functions Reference, 2012). Recognition memory occurs when the area is activated by an auditory task as well as a visual task (Hugdahl & Davidson, 2004). The HIV/AIDS group in this current study compared to the control group showed greater activation in voxels in the BA37 area. Ardila et al. (2016) suggest that, although BA20 and BA37 are part of language processes, it cannot be considered a core receptive language processing area (such as Wernicke's area). There is a fringe, or peripheral, zone around this core (Wernicke's) area involved in language associations. This fringe zone corresponds to BA20 and BA37 (Ardila et al., 2016). In the current study BA37 and BA20 areas were activated in the HIV/AIDS group, although BA20 had a higher voxel count than BA37. BA37 can be involved in associated language functions, memory circuitries, word retrieval, attention, word generation, and visual processing (Cortical Functions Reference, 2012).

A recent study by Zhan, Buckey, Fellows, and Shi (2017) showed that HIV/AIDS affects several areas of the brain that are involved in central auditory processing, particularly the thalamus, internal capsule, and temporal cortex. The thalamus contains the medial geniculate body, and this finding may be relevant for auditory processing.

The precuneus is the part of the parietal lobe that is associated with visual motor coordination and working memory (motor, visual, auditory, emotional, and verbal). This area is also part of the complex involved in proxemics, that is, the perception of personal space around a person (Cortical Functions Reference, 2012). The activation regarding proxemics occurs in the following regions: the parietal lobe and precuneus region (spatial perception). The cingulate gyrus and the sub-lobar region (lentiform nucleus, caudate, insula, and thalamus) are responsible for error monitoring and control of unnecessary movements (Choi, Kim, Yoon, Lee, J. Baek, Choi & Chung, 2017). In the current study only passive listening was investigated, and no motor coordination or specific function of an area was monitored.

In addition, the HIV/AIDS group with HAND showed a decline in brain activation patterns, suggesting a diminished reserve capacity for compensation in this group. Thus, functional studies suggest activation in the CANS area with additive or synergistic effects of HIV/AIDS with abnormalities in the CANS area.

5.7. Comprehensive conclusions

This chapter provided the reader with the discussion of the results, which were presented in Chapter 4. It highlighted the most important findings and placed the findings within the context of existing literature. fMRI measures the hemodynamic response of the brain to changes in neural activity. De Lange (2007) concluded that individuals with HIV/AIDS do not always show observable auditory manifestations or loss of hearing, although HIV/AIDS can have an influence on the neurological pathways of the auditory pathway, even in the early stages of HIV/AIDS. Determining the response of the CANS to sound in persons with HIV/AIDS could ultimately contribute to better diagnosis and a better service delivery.

Firszt, Ulmer and Gaggl (2006) conducted a review of central auditory deficits of participants with HIV/AIDS. These may involve neurological defects in the pathways from the auditory nerve through to the higher auditory pathways in the brain. The review showed that HIV/AIDS affects several areas in the brain, in particular the thalamus, internal capsule, and temporal cortex, all of which are involved in the CANS. These findings confirm that HIV/AIDS can affect the central auditory pathways and support the potential use of central auditory tests as a way to assess the effects of HIV/AIDS on the CANS.

ROIs were identified to determine the activation in the CANS areas in response to auditory stimuli. The results of the activation of the ROIs that were determined (aim 1 and aim 2) confirmed the similar regions of activation in the two groups of participants. The whole brain analysis was done to evaluate the response of the whole brain to auditory stimuli. The whole brain analysis (aim 3) illustrated the utility of fMRI for examining particular regions of interest within the brain to determine whether there are abnormal changes in structure both over time in the participants with HIV/AIDS, and in comparison to controls.

Figure 30 summarises the findings with regard to the aims using an auditory and language processing continuum model.

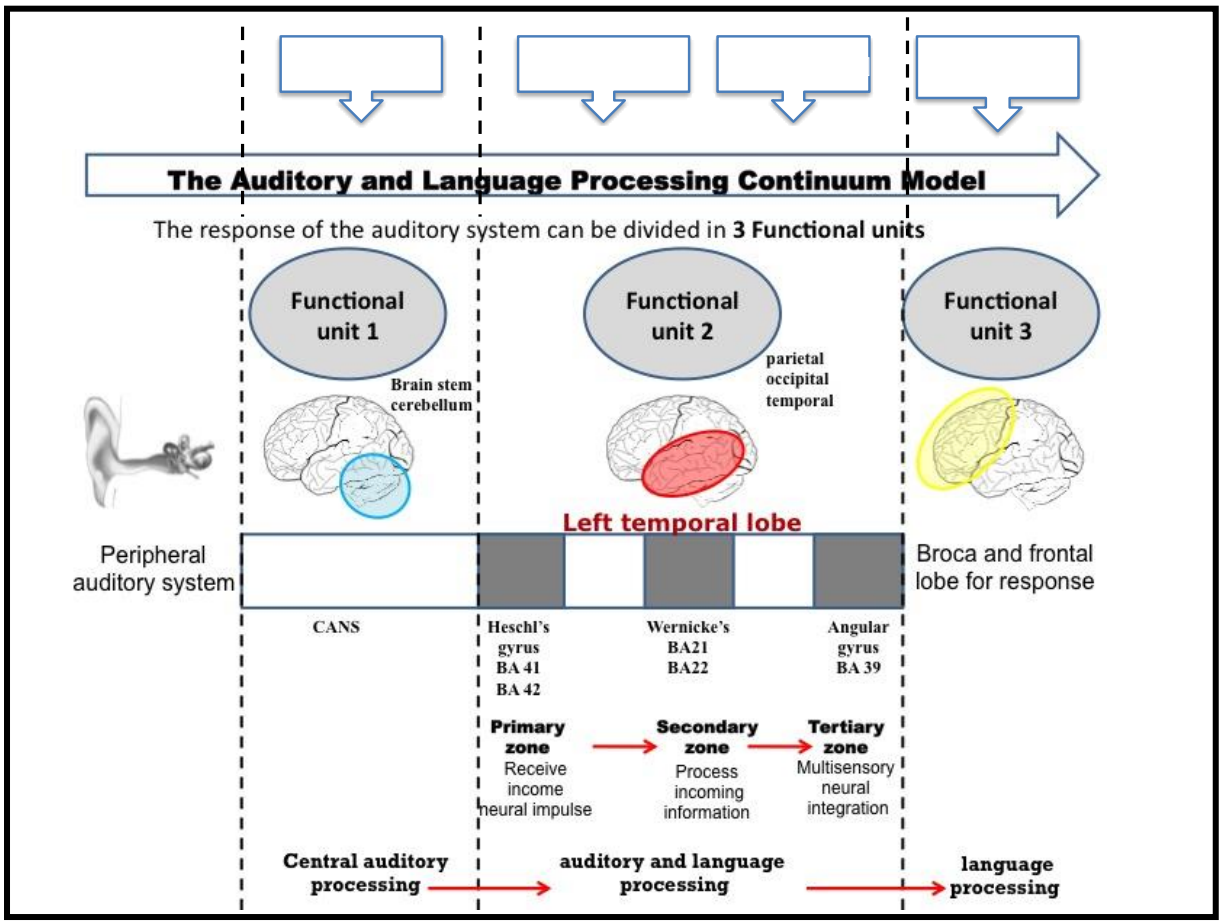


Figure 30: Auditory and language processing continuum model showing where the aims fit in

The response in the auditory system can be divided into functional units:

- Functional unit 1

  The scope of results of sub-aim 2 can apply to Functional unit 1, which is part of the CANS and is involved in central auditory processing, which takes place in the brainstem and cerebellum area.

  For sub-aim 2 the results indicated a marginally significant difference between the HIV/AIDS group and the control group with regard to the CANS (ROI) response to warble tones. The HIV/AIDS group had lesser activation in the right inferior colliculus than the control group. This negative response of the BOLD mean percentage signal

change reflects a decrease in cerebral blood flow (decrease in oxygen consumption and neural activity) which can be induced by a reallocation of blood flow from the less demanding areas to the most demanding regions in the brain. These findings relating to sub-aim 1 and sub-aim 2 suggest that the nonsense syllables and warble tone tasks appear to be suitable for auditory fMRI and also that fMRI is suitable to detect BOLD signal change in the specific ROIs connected to the central auditory system.

- Functional unit 2

  With regard to sub-aim 1 a significant difference in the percentage mean signal change was obtained between the two groups of participants for both BA41 and BA42. Both BA41 and BA42 are situated in Heschl's gyrus, as part of the primary auditory cortex. The control group activation was greater than for the HIV/AIDS group. Heschl's gyrus is the intersection area where auditory processing (auditory stimulus) and language processing (meaning) occur. The control group showed a significantly greater activation bilaterally than the HIV/AIDS group in these areas, with a significantly greater increase in neuronal activity caused by cerebral blood flow to BA41 and BA42 than the HIV/AIDS group.

  Sub-aim 3 required comparison of the response of the whole brain to nonsense syllables and warble tones in participants with normal hearing with and without HIV/AIDS. Several results are applicable to functional unit 2. In the nonsense syllables task both groups had activation in BA41 compared to the baseline activity, although the control group had more activated voxels than the HIV/AIDS group. In the warble tones task both groups showed differences compared to baseline. The control group showed greater activation in the sub-lobar lobe and the HIV/AIDS group showed greater activation in the temporal lobe. When compared to the HIV/AIDS group, the control group showed more activation in the sub-lobar area. When compared to the control group, the HIV/AIDS group showed more activation in the parietal lobe. The results of the whole brain analysis showed that the different participant groups differ in brain activation and confirmatory voxel-by-voxel comparison results were noted. HIV/AIDS can affect the CANS.

  The results relating to both sub-aim 1 and sub-aim 3 involve the auditory and language processing that takes place in the parietal, occipital, and temporal lobe.

- Functional unit 3

  Sub-aim 3 encompasses the language processing that takes place in the frontal and Broca areas of the brain. Only the control group showed activation in the frontal lobe of the brain.

The fMRI data revealed that the nonsense syllables and warble tone tasks activated similar brain regions in the two groups within the auditory ROIs that were examined and showed comparable timing of activation. fMRI measures brain activity by detecting changes associated with blood flow. Blood flow to the activated area reflects increased metabolic activity, which implies function of that area. Auditory stimulation activates the left hemisphere where Wernicke's area, the area important for listening to words, is situated.

If warble tones were presented to the participants, their right hemisphere was active. This leads to the conclusion that the two types of stimuli are processed differently by the brain (Seikel, Drumright & King, 2015). Hemispheric asymmetries were affected by a number of variables including stimulus type, the presence of pathology, and the individual's characteristics. Although it appears that the right and left hemispheres are not identical in structure or function, and that there is a correlation between anatomic, structural, and functional asymmetries, there is much to be learned about the differential representation of speech sounds in human cerebral hemispheres. Neuroimaging techniques, such as fMRI, will accelerate our understanding of the human auditory cortex and the relationship between structure and function.

Zhan et al., (2017) reported a systematic review of the literature to evaluate the evidence for HIV/AIDS affecting parts of the central nervous system involved in central auditory processing. These findings support the idea that HIV/AIDS can affect the central auditory pathways and support the use of central auditory tests to assess the central nervous system.

The importance to non-invasively evaluate human auditory pathway function on a 3-D map basis was only made possible by the advent of functional neuroimaging methods. The purpose of measuring the evoked potentials, became the first neuroimaging technique to be directed at the study of human auditory function. Evoked potentials have historically provided only limited information about the structure and organising principles of the central auditory pathway. Evoked potentials have not proven useful for non-invasive clarification of the

auditory processing process at a resolution below the level of the nucleus or a prominent, damaged, cell population in contrast to fMRI.

fMRI have significantly contributed to the understanding of the neurosciences to obtain functional dynamic, time-varying information (Talavage, Gonzalez-Castillo & Scott, 2014).

Functional MRI is a useful additional diagnostic tool, therefore, and the results obtained with the use of fMRI were complementary to the other observations. Further studies involving the role of fMRI are needed to evaluate in what way auditory fMRI data might vary or differ from data obtained by other means with regard to the main characteristics of HIV/AIDS. Our paradigm adequately visualized the central auditory pathways in both the control and HIV/AIDS participants. In this study, activation in the cortical and subcortical area was successfully visualized with auditory fMRI activation. The auditory system in both the control participants and the participants with HIV/AIDS was successfully visualised using auditory fMRI.

From the discussion, the current predicament becomes clear: HIV/AIDS can affect the CANS, but these changes have not been explored sufficiently and should therefore be further investigated and documented.

CHAPTER 6
CONCLUSIONS, CLINICAL IMPLICATIONS, AND RECOMMENDATIONS

"*Great is the art of beginning, but greater the art is of ending*"
(Henry Wadsworth Longfellow)

In this study, auditory fMRI was used to investigate the response of the CANS in individuals with and without HIV/AIDS, using two different auditory stimuli. BrainVoyager Software was used to analyse and visualise the functional and structural aspects of the brain regions (spheres) defined as ROIs, namely the CN, SOC, IC, MGN, BA41 and BA42. Whole-brain analysis, using a map of new functional brain regions, was employed to characterise further how known specialized brain areas would respond to nonsense syllables and warble tones in the two different groups of participants. The conclusions, limitations of this study, clinical implications, and recommendations for future research are discussed in this chapter.

6.1. Conclusions

The results obtained from this study suggest several important conclusions. The results are discussed in accordance with the aims of the study.

6.1.1. Results relating to sub-aim 1:

In order to explore the data concerning the response of the CANS to nonsense syllables, it was necessary to perform ROI analysis of the CANS areas in the brain. Both groups of participants showed activation in all the ROIs of the CANS, but the mean percentage signal change in BA41 and BA42 for the HIV/AIDS group and for the control group differed significantly. There was a significant difference in activation for nonsense syllables in both sides of the brain, and this was observed in both BA41 ($p = 0.03$) and BA42 ($p = 0.03$) ROI's. The control group showed a significantly greater activation bilaterally than the HIV/AIDS

group in these areas, that is to say, the control group showed a significantly greater increase in neuronal activity caused by cerebral blood flow to BA41 and BA42 than the HIV/AIDS group.

The influence of possible confounding factors such as gender, age, and education on the mean percentage signal change in BA41 and BA42 in the two groups was also determined. Age ($p = 0.95$) and education ($p = 0.68$) had no statistically significant effect on the mean percentage signal change in BA41 and BA42 for any of the two groups in this study. However, gender had a significant effect for the mean percentage signal change for BA41 ($p = 0.042$) and BA42 ($p = 0.037$) with a p-value of 0.04. With regard to the independent variables gender and group, there was no significant difference between the male participants of the two groups ($p = 0.614$), but a significant difference was found between female participants ($p = 0.008$). The mean percentage signal change (M) for the areas BA41 and BA42 was determined to be $M = 0.088$ in the HIV/AIDS group and $M = 0.170$ in the control group. The interaction between the gender of the participants and the two groups (HIV/AIDS and control) showed a significant difference in female participants of this study. It is noteworthy that Torre et al. (2015) found that female participants with HIV/AIDS had more severe hearing loss than male participants with HIV/AIDS.

In Chapter 5 it was mentioned that Burman, Bitan and Booth (2008) found stronger activation of the cortical language processing areas in females than in males. In the current study the control group females had better mean percentage signal change in BA41 and BA42 than the male participants. However, the female population may be influenced more by HIV/AIDS than their male counterparts. Research concerning HIV/AIDS, cognition and women (Maki & Martin-Thormeyer, 2009) found that women with HIV/AIDS showed a significantly higher prevalence of neurocognitive impairment than women who did not have HIV/AIDS. In the current study the males with HIV/AIDS had significantly higher mean percentage signal change in the areas of BA41 and BA42 than the females with HIV/AIDS.

It was also considered relevant to determine whether the CD4 count has any effect on the neural response of the ROIs CANS to the auditory stimulation namely nonsense syllables task in the HIV/AIDS group. With regards to the CD4 count, the effect of the CD4 count and years in ART could have an influence on the findings relating to BA41 and BA42. The results showed a negative linear regression analysis with inverse correlations between the CD4 count and ART use in years, and the mean percentage signal change in BA41 or BA42.

As either the CD4 count or the years of use of ART increases, the mean percentage signal change in BA41 or BA42 decreases. This negative response to the mean percentage signal change reflects a decrease in oxygen consumption and neural activity following auditory stimulation, which can be induced by a reallocation of blood flow from the less demanding areas to the most demanding regions in the brain. The HIV/AIDS disease severity in terms of the CD4 count can be related to ART use in years. In this study an inverse correlation was noted between the years on ART and the activated BOLD signals in BA41 and BA42 areas during the nonsense syllables task. The use of ART could have reduced the CD4 count in this study. Although this tendency was observed, it would require a bigger sample to shed light on these findings.

6.1.2. Results relating to sub-aim 2:

With regards to the response of the CANS (ROI's) to warble tones no significant differences between all the ROIs between the two groups where found. The only ROI where a borderline significant difference of activation occurred, was the inferior colliculus in the right hemisphere of the HIV/AIDS group. The difference in the activation of the right inferior colliculus between the two groups was borderline significant (p=0.07), with the control group showing a higher mean percentage signal change (M = -0.002), than the HIV/AIDS group (M = -0.046).

This negative response of the BOLD mean percentage signal change reflects a decrease in cerebral blood flow (decrease in oxygen consumption and neural activity) which can be induced by a reallocation of blood flow from the less demanding areas to the most demanding regions in the brain.

These findings relating to sub-aim 1 and sub-aim 2 suggest that the nonsense syllables and warble tone tasks appear to be suitable for auditory fMRI and also that fMRI is suitable to detect BOLD signal change in the specific ROIs connected to the central auditory system.

6.1.3. Results relating to sub-aim 3:

Whole brain analysis was used to examine the particular regions of interest within the brain in order to determine whether there were abnormal changes in the structures of the brain over time in the groups separately and also in comparison with each other. The participants in both the control group and the HIV/AIDS group showed increased voxel activation in certain regions of the brain compared to baseline, as expected in response to the auditory stimulation namely nonsense syllables and warble tones. In summary the results of the whole brain analysis showed that the different participant groups differ in brain activation and confirmatory voxel-by-voxel comparison results were noted. HIV/AIDS can affect the CANS, but the changes that were observed were not explored in depth and should therefore be investigated and documented further.

6.2. Limitations

A discussion of the limitations of this study can be used to improve on the current research and help shape the future research agenda. Discussing the implications of possible limitations prevents misunderstandings and supports the interpretation of data. Instead of perceiving the acknowledgment of limitations negatively, researchers/authors should recognise the potential value of a truthful and unbiased discussion of study limitations. In addition, more research is needed to see how the acknowledgment of potentially important limitations fits with the claims made. The importance and validity of a study is not diminished, and false accusations can be prevented.

- fMRI is an expensive, time consuming, and labour-intensive technique, therefore it cannot currently be used routinely as part of standard evaluations of the CANS. It remains useful for brain research purposes and certainly with new technology and methodological development fMRI will most definitely play an important role in the development of new techniques and clinical implications in the future.
- Due to the limited access to patients who attended the clinic at the time of the study, the researcher was unable to recruit a larger sample for the control group to be able to match the two groups with regard to sample size, age, and gender.

- fMRI seems a potentially important tool for the understanding of HIV/AIDS. Nevertheless, much more research will be necessary before fMRI can serve as a diagnostic method to classify various types of auditory pathology. fMRI can, however, pinpoint the location of dysfunctional areas in the brain.

6.3. Strengths and clinical implications of the research findings

A discussion of the strengths of this study can be used to set an appropriate quality of standard. The strengths highlighted here relate mainly to the research tool.

- The application of fMRI to the CANS was shown to have promising potential. Research applying fMRI to evaluate activation of the CANS is currently limited.
- fMRI is likely to become the tool of choice for addressing many research questions concerning the auditory system, especially regarding the localization of the function of the auditory areas and brain connectivity.
- fMRI has a good spatial sensitivity and specificity and can be used to map auditory responses in detail as well as for localization of the responses.
- fMRI is non-invasive and is therefore suitable for research involving both children and adults.
- Multiple observations can be made on the same individual in respect of specific brain structures. fMRI permits the investigation of longer term processes such as treatment, intervention, and rehabilitation, and allows comparison of results obtained at different times.
- fMRI will remain an essential tool for brain research purposes, especially in the light of all the new technological and methodological developments that have only started to emerge. Therefore, fMRI will probably play an important role in the development of new techniques for clinical application in the near future.
- A better understanding has been achieved of how HIV/AIDS affects the auditory part of the brain, but there is still much to be learned about how HIV/AIDS affects the brain.

Clinical audiologists typically use audiometric testing for the diagnosis of hearing impairment. None of the current guidelines recommend imaging testing. Resourceful clinicians have, however, taken note of the fact that techniques have been developed which enable researchers to visualise the brain and how it functions. As evident from this research, fMRI may be used more often in research and in clinical practice to visualise the activity of the auditory pathways and to identify abnormalities in the pathway.

BOLD fMRI provides a possibility to display brain regions responding to specific auditory stimuli applied during the scanning session. It has become an essential tool for studying human auditory function. In the current study differences in activation were observed in participants with HIV/AIDS compared to participants without HIV/AIDS. These differences could not have been observed with any other basic audiometric test procedure in people with HIV/AIDS.

The role of fMRI in a clinical context is as yet limited, although it is becoming increasingly important for mapping brain functions in individuals (especially for demarcating speech related regions) prior to surgical treatment of brain tumours. As more detailed knowledge is obtained about the various functions of the brain, more clinical applications of fMRI may emerge (Hennig, Speck, Koch & Weiller, 2003).

The current research findings will no doubt be a valuable starting point for future research projects within the field of audiology. It is envisaged that fMRI will probably play an important role in the development of new techniques for clinical application in the near future.

6.4. Recommendations for future research

HIV/AIDS remains a growing epidemic and a worldwide concern. Numerous resources are being employed to control the spread of the disease and to prevent mortality due to the disease and its manifestations.

The life expectancy of individuals infected with HIV/AIDS has increased due to the use of antiretroviral treatment measures. This effectively poses challenges for the management and maintenance of the quality of life of individuals living with HIV/AIDS today. Various new

research questions emerged from this study and recommendations for future areas of research are listed below.

- Various indicators of auditory dysfunction due to HIV/AIDS have been identified. Current knowledge in this field of research, however, still remains limited. It is the responsibility of hearing care researchers to expand the current understanding of HIV/AIDS related auditory manifestations to ensure that individuals infected with HIV/AIDS ultimately have access to preventative hearing health care. This is especially important due to the lifelong nature of HIV/AIDS, the significantly increased life expectancy due to highly active antiretroviral treatment, and subsequently the increasingly growing population living with HIV/AIDS and its auditory manifestations.
- This study has shown that other professions (radiology) can improve the understanding and knowledge of auditory dysfunction when fMRI is used in combination with standard audiometric procedures. Collaborative practice should be a primary area of research.
- Judging from the insight gained from functional imaging research with normal hearing participants in the current study, it would be efficient to study participants with a variety of hearing impairments.
- The use of MRI in a clinical environment, and in an audiology department in particular, is currently mostly limited to the imaging of purely anatomical features. It is becoming increasingly important, though, for mapping brain functions in individuals (especially for demarcating speech related regions) prior to surgical treatment of brain tumours. As more detailed knowledge is obtained about the various functions of the brain, more clinical applications of fMRI may emerge. In the field of auditory processing a number of potential applications can be envisaged for future clinical use.

Functional imaging techniques afford researchers an indirect view of the auditory activity of the brain in humans. These techniques are non-invasive, and it is possible to determine auditory-related activity throughout the whole brain over a given period of time. Although functional imaging of the CANS has not been widely used in ear, nose and throat or audiology departments, a growing literature on its potential application is being developed. Insights gained from functional imaging research with normal-hearing subjects can help to develop efficient protocols to study patients with hearing problems.

Research concerning the identification, assessment, and the effect of auditory pathology and treatment protocols should be put in place and be made available to equip the health sector for accountable service delivery to all patients. The compilation and evaluation of a research based systematic assessment and management protocol for hearing healthcare professionals as part of the multi-disciplinary team for the management of individuals living with HIV/AIDS is recommended.

The inclusion of audiologists and radiographers in a multi-disciplinary team may contribute to the prevention and earlier detection of auditory disorders in the HIV/AIDS community. Routine hearing tests are essential in order to track the hearing health of the HIV/AIDS population. Early intervention may increase their life quality. Larger and more analytic studies on the effect of HIV/AIDS and ART on the auditory system are still needed to better understand the challenges and improve care and treatment.

6.5. Final comment

The confirmation of our hypotheses adequately supports the value of visualization of the activation in both cortical and subcortical structures of the auditory pathways in participants with HIV/AIDS and the control participants. The findings support the alternative hypotheses for sub-aim 1, sub-aim 2 and sub-aim 3. Although with reference to sub-aim 2 the right inferior colliculus showed a borderline significant difference (p=0.07), with the mean percentage signal change being higher in the control group (M = -0.002) while the HIV/AIDS group showed a lesser mean percentage signal change (M = -0.046), the alternative hypothesis was nevertheless confirmed.

The alternative hypothesis was confirmed for all the aims:

- ✓ **$H1_a$:** There is a **difference** between the response (as measured with fMRI) of the CANS (ROI) to **nonsense syllables** in normal hearing adults with HIV/AIDS and in normal hearing adults without HIV/AIDS.

- ✓ **$H2_a$:** There is a **difference** in the response (as measured with fMRI) of the CANS (ROI) to **warble tones** in normal hearing adults with HIV/AIDS and in normal hearing adults without HIV/AIDS.

- ✓ **$H3_a$:** There is a **difference** in the response of the whole brain to nonsense syllables and warble tones in participants with and without HIV/AIDS.

The main aim of the study - to determine the response of the central auditory nervous system (CANS) to sound in normal hearing adults with and without HIV/AIDS, using fMRI - as well as the sub-aims was achieved using different hearing tasks. In all the subsections of research the areas of activation were found in the CANS areas.

fMRI of the auditory system is a new research frontier for clinicians involved in the field of audiology and radiology. There has been an explosive interest and rapid pace of development in the use of fMRI. Within the last decade, technological advancements have made fMRI more accessible for application to auditory neuroscience. fMRI based on BOLD contrast has gained a primary role in the study of the human brain, both for characterisation of normal brain activity and for clinical practice. Although there is a considerable amount of research dealing with activation of the auditory cortex, relatively little information exists on functional imaging of the subcortical auditory pathway. The auditory system consists of multiple processing centres distributed across the medulla, pons, midbrain, thalamus, and temporal cortex. fMRI is a powerful technique because of the high spatial resolution and also because it is a non-invasive method to study and evaluate these areas.

Obtaining a better and more specific diagnosis may assist in developing more appropriate and evidenced-based rehabilitation strategies for this population. It will also be of great value in countries where the incidence of HIV/AIDS is high, as in South Africa. To date, not enough auditory fMRI research has been performed to determine the influence of HIV/AIDS on the CANS. fMRI can highlight those areas that are abnormal or have no neural activity, and should contribute positively to the field of auditory examination and the understanding of the consequences of HIV/AIDS (Khoza-Shangase, 2010)

Clinicians should distinguish to the best of their knowledge between symptoms related to the HIV/AIDS disease process and the side effects of ART. Numerous factors related to HIV/AIDS can influence communication abilities. These factors can include CNS abnormalities related to the infection, opportunistic infection, and treatment effects. Assessments of communication disorders should be obtained as necessary to provide quality care to people with HIV/AIDS and to maintain the quality of life that effective communication provides (Kallail, Downs& Scherz, 2008).

The role of the radiographer is constantly developing and expanding into new areas of responsibility. As an MR radiographer, I wish to develop my career in management, career support and developing, teaching, and research. I believe that the role of a radiographer does not begin and end in the x-ray department but can be much more extensive. The findings of the current research, in addition to their obvious value, also contribute by pointing to the benefits of combining professionals from a variety of fields to form a transdisciplinary research team. It will be necessary to focus on linking theory to practice for intervention for people with HIV/AIDS. fMRI could supply answers to questions posed in different career fields, whereas each career field could provide important concepts that can assist in improving the scope and efficacy of auditory fMRI research.

"The tipping point for African research innovation will not be merely the ability to fully access and use new abundance of global knowledge and ideas but to make an active and significant contribution to its creation"

(Piyushi Kotecha)

"*After climbing a great hill, one only finds that there are many more hills to climb.*"

~ Nelson Mandela

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APPENDICES

APPENDIX A: INFORMATION LEAFLET

PARTICIPANT INFORMATION

Functional Magnetic Resonance Imaging (fMRI) of the auditory system

1 WHAT IS AN MRI?

MRI stands for Magnetic Resonance Imaging, which is a special scan that uses very strong magnet and radio waves to take pictures of the inside of your body.

2 WHAT IS AN fMRI OF THE BRAIN?

fMRI stands for functional Magnetic Resonance Imaging, which is an MRI scan of the brain that measures the blood flow to the active areas of the brain.

fMRI is a method to learn how the brain works.

3 IS fMRI SAFE?

Yes, there is no exposure to x-ray radiation during the fMRI examination. Because the MRI scanner is a very strong magnet, it is very important to screen people to check whether they have metallic and magnetic implants or items. You will be asked to complete a detailed form for this purpose.

Remember to remove all metal objects before entering the MRI room, including cell phones and bank cards.

Inform the radiographer if you have any metal implants or a pacemaker

4 PREPARATION

You will be asked to remove all metallic objects (watches, jewellery, dentures, hairpins, cell phones and money) and lock these in a cupboard. You will need to change into a gown.

5 CLAUSTROPHOBIA

If you suffer from extreme claustrophobia the scan may induce some anxiety. If you are able to use an elevator in a shopping centre/hospital, the scan should be no different.

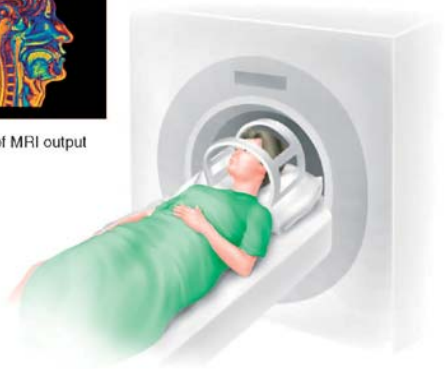
6 PROCEDURE

When the MRI scanner is working it is very noisy and makes tapping and banging sounds. This is how the MRI scanner works to form pictures.

You will be provided with headphones to protect your ears.

During the examination you will lie on a padded table which slides into the machine.

Example of MRI output



The MRI “tube” is open at both ends. The inside of the “tube” has a light and is well ventilated for your comfort.

Foam cushions are used to help you keep still as you must not move during the procedure. A special frame, which looks like a helmet will be placed over your head. This assists to receive the pictures.

All you need to do is relax and lie as still as possible.

In an fMRI examination, you will perform a particular task during the imaging process, causing increased activity in the area of the brain responsible for the task. Your task will be to close your eyes and listen to the sounds through the headphones.

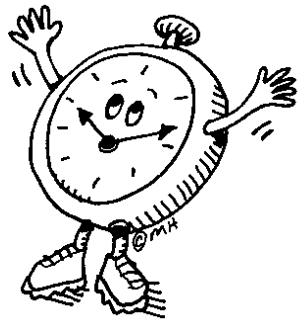
This activity, which includes expanding blood vessels, chemical changes and the delivery of extra oxygen, will then be recorded on MRI images.

7 WHAT ARE THE ADVANTAGES OF MRI

MRI is “non-invasive” the MRI does not use x-rays, so there is no exposure to ionizing radiation.

8 WHAT TO EXPECT DURING YOUR EXAMINATION & HOW LONG WILL IT TAKE?

The fMRI scan is painless and takes about 30 minutes. There is nothing to be afraid of.



9 THE VALUE OF THE fMRI STUDY

Obtaining this information will help to appropriately diagnose hearing problems due to central auditory involvement as well as ensure that these patients receive suitable rehabilitation for their hearing disorders.

Pictures obtained from:

- www.dreamstime.com
- http://familydoctor.co.uk/media/upload/MRI.jpg
- www.tecnocem.com/.../auditory-protection.jpg
- www.jumbletown.ie/forums/attachment.php?attachmentid=775&stc=1&d=1158874490
- http://img109.imageshack.us/img109/4742/fmri01js5.jpg
- http://bw.stma.k12.mn.us/resources/2nd_grade/_image/clock.gif

Information obtained from:

- http://radiologyinfo.org/en/info.cfm?pg=fmribrain

This pamphlet has been designed by Celesté Pretorius

APPENDIX B: INFORMED CONSENT LETTER

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

1 MILITARY HOSPITAL
Department of ENT
Tel: +27 12 314 0443
Fax : +27 12 314 0446
Email: lmhofmeyr@surgeon.co.za

Faculty of Humanities
Dept of Communication Pathology
Speech, Voice and Hearing Clinic
Tel: +27 12 420 2304
Fax : +27 12 420 3517
Email: maggi.soer@up.ac.za

11 March 2013

Dear Participant

REQUEST FOR YOUR PARTICIPATION IN A RESEARCH PROJECT

I am a qualified MR Radiographer and DPhil student in Communication Pathology at the University of Pretoria. It is expected of me to conduct a research project in partial fulfilment of the requirements for my degree. The title of my study is: *The response of the central auditory nervous system to sound in normal hearing adults with and without HIV/AIDS: An fMRI study*. I would appreciate it if you would be willing to participate in this project.

Purpose of the study:

This study is about hearing disorders and the possibility that HIV/AIDS may contribute to a hearing problem in the central auditory nervous system (part of the brain). Participants with and without HIV/AIDS will be involved in the study. The information obtained from this study may be used to determine the extent of such hearing disorders. During the study, fMRI examinations will be done to obtain information that may help clinicians to establish whether HIV/AIDS causes this type of hearing disorder.

Procedure:

You are requested to participate in various non-invasive hearing tests and an fMRI scan procedure. The fMRI examination is simple, safe and painless. The hearing tests will be done at 1Military Hospital and the fMRI examination at the Meulmed Hospital. A pamphlet is included with this letter to explain the fMRI examination.

Risks and possible discomforts:

There are no risks involved in this study. It will be expected of you to attend the fMRI procedure on the arranged date and time. Because of the strong magnet during the procedure, metal objects in your body may be hazardous and therefore not allowed in the MRI department. If you suffer from extreme claustrophobia the scan may induce some anxiety. If you are able to use a lift in shopping centre/hospital the scan should be no different.

Value of the study:

By participating in this study, you will contribute to the results on the effect of HIV/AIDS on the central auditory nervous system (a part of the brain). Nowhere in the world has this research been done before. This study will be truly groundbreaking and unique as no research nationally or internationally has been performed to determine the influence of HIV/AIDS on the central auditory system. Obtaining this information will help to appropriately diagnose hearing problems due to central auditory involvement as well as ensure that these patients receive suitable rehabilitation for their hearing disorders.

The results obtained from this project will therefore be of great value in countries where people struggle with HIV/AIDS.

Compensation & Costs:

You will not be paid to participate in the study, but all your travel expenses will be adequately reimbursed. You will have no other additional expenses/costs towards the study. The hearing tests and fMRI examination will be done at no cost to you.

Participant's rights:

You are requested to participate voluntarily in this study. You have the right to withdraw from participation in the study at any time without negative consequences.

Participant's personal medical information:

Should you decide to participate in this research project, you will undergo various non-invasive procedures. It also involves the researcher studying your medical files for information regarding your health and blood tests that were taken at the clinic. You will also undergo a medical examination performed by a qualified medical doctor. The information from your personal file will be used to assist the researcher to investigate the research question and your permission to utilize your file is therefore requested.

Confidentiality:

All information collected in the study will be treated in a confidential manner and will be used only for medical research and reported to the appropriate authorities, always respecting confidentiality (your name and personal details will **not** therefore be used). No-one else will know whether you have a hearing disorder or whether you are HIV positive or not. Your participation in this study will not influence you, your relationship with your friends, family or your colleagues at work because they will not be aware of this or know the results of the various tests. You can be assured that any information that you have provided will be destroyed should you choose to withdraw from the study. By signing the consent to take part in this study, you are authorizing access to your clinical data by Celeste Pretorius, research audiologist and medical personnel of the University of Pretoria working specifically with this research trial, Research Ethics Committee of the Faculty of Humanities of the University of Pretoria and the South African Military Health Services Research Ethics Committee. This is standard requirements for all clinical studies. By signing this letter you are authorizing such access to your medical records. When the results of the study are published at a later date, all these results will be dealt with in strict confidentiality and your identity will **not be** disclosed. Confidentiality of all personal details will be maintained at all times.

Ethics Approval:

The protocol of this clinical study was submitted for approval to the Research Ethics Committee of the Faculty of Humanities of the University of Pretoria and the South African Military Health Services Research Ethics Committee (contact details can be obtained from the researcher). These research ethics committees are registered with the National Health Research Ethics Council. Written approval has been granted by the Research Ethics Committee of the Faculty of Humanities of the University of Pretoria and the South African Military Health Services Research Ethics Committee to conduct the study. The study has been structured in accordance with the Guidelines on Clinical Trials and Ethics in Health Research, published by the Department of Health and the Declaration of Helsinki (last updated October 2000, including October 2004 Notes of Clarification), adopted by the World medical Association (WMA), which deals with the recommendation guiding doctors in biomedical research involving human participants. Copies of these documents may be obtained from the researcher should you wish to review it.

Dissemination:

The summary of results will be available to subjects on request. The information and results of this research project will be available in format of a thesis at the library of the University of Pretoria as well as in an article publication and presentation. All raw data will be stored in hard copy on CD for 15 years before it will be destroyed.

If you need further information or have any questions or concerns, you are welcome to contact me, Celesté Pretorius, any time at 082 4191749.

Kind regards,

Celeste Pretorius

INFORMED CONSENT

I, …… declare that I have been informed by the researcher, regarding the nature, conduct, benefits and risks of the clinical trial.

I understand that my name and personal details will not be disclosed in any report.

- o I confirm that I have read and understood the above written information in this information and consent letter
- o I have been given the opportunity to ask questions about the study procedures to clarify any misunderstanding.
- o I give permission to the Research Ethics Committee of the Faculty of Humanities of the University of Pretoria and the South African Military Health Services Research Ethics Committee to have access to my medical records when required for source Document Verification.
- o I understand that I am entitled to receive a signed copy of this document.

Research Participant

_____	_____	_____
Print name	Signature	Date

I hereby confirm that the above patient has been informed fully about the nature, conduct and risks of the above trial.

Researcher

_____	_____	_____
Print name	Signature	Date

Witness

_____	_____	_____
Print name	Signature	Date

Witness signature confirms that he/she has witnessed the relevant signatures at the time of signing. Witness name, signature and date must be completed by the witness at the same time that this document is signed and dated by the patient and the researcher.

APPENDIX C: CASE REPORT FORM

Participant number _____

Control group	
Exploratory group	

The response of the central auditory nervous system to sound in normal hearing adults with and without HIV/AIDS: An fMRI study

CASE REPORT FORM

Case Report Form to be completed by Principal Investigator and Co-Investigators.
*Make use of "**X**" to mark the relevant answers*

Principal Investigator	**Celesté Pretorius**

SECTION A: BIOGRAPHICAL INFORMATION

Completed by: _____

Date (dd/mm/yyyy) _____

Participant number _____

Date of Birth (dd/mm/yyyy) _____

Gender

Male	
Female	

Age _____

Weight _____ kg

Home Language _____

Educational Level _____

SECTION B: MEDICAL HISTORY*

Completed by: _____

Inclusion Criteria (Must be "YES" to include Research Participant)

HIV/AIDS

YES	
NO	

Date of last test (dd/mm/yyyy) _____

HIV/AIDS Categories

Clinical category of HIV/AIDS

A	B	C

	YES	**NO**
CD4+ cell count less than 3 months		
Category 1: >500 CD4+ T-cells/µl		
Category 2: 200 - 499 CD4+ T-cells/µl		
Category 3: <200 CD4+ T-cells/µl		

History of TB treatment		History of medical therapy (ART)	
Treatment	How many times	Treatment	How many times
Regime I		ARVD	
Regime II (Streptomycin)		No Treatment	
MDR (Amikacin & Streptomycin)		ART treatment commenced on	
No History			

* Medical History will be confirmed by blood test

SECTION C: MAGNETIC RESONANCE IMAGING (MRI) COMPATIBILITY

Completed by: _____

Exclusion Criteria (Must be "NO" to include Research Participant)

MRI suitability	**YES**	**NO**	**EXCLUSION**
Female - **Pregnant**			
Cardiac pacemaker			
Heart or chest:			
- Heart valve			
- Carotid clip			
- Venous umbrella			
- Bypass VP shunt or heart shunt			
Head or brain:			
- Aneurysm			
- Programmable hydrocephalus shunt			
Eyes:			
- Metal objects			
- Metal splinters			
Ears:			
- Cochlear implant			
- Ear prosthesis			
Operations:			
- Metal implant			
- Artificial limb			
- Joint replacement			
- Shrapnel			
- Prosthesis			
- Neurostimulator			
- Insulin pump			
Claustrophobia			

Information verified by a MRI Radiographer

MRI Radiographer Signature _____ **Date** _____

Adapted from: Dr's de Beer, de Jager Radiologists Arcadia MRI Questionaire.

SECTION D: CLINICAL EXAMINATION OF AUDITORY SYSTEM

Completed by: _____

Exclusion Criteria (Must be "No" to include Research Participant)

Otological complaints currently or the last 3 months that may exclude the participant from the study

Symptom	Yes	No	Describe if "Yes"	Exclusion
Hearing loss				
Vertigo				
Tinnitus				
Otalgia				
Otorrhea				
Otitis media				
Grommits				

Exclusion Criteria (Must be "No" to include Research Participant)

The participant's disease spectrum that presents with one of the following disorders that may effect the participant's inclusion. Does participant present with the following or had a once off insidence of the following:

Symptom	Yes	No	Describe if "Yes"	Exclusion
Meningitis				
Encephalitis				
Convulsions / Epilepsy				
Facial nerve palsy				
Cerebal palsy (CP)				
Dysphagia				
Voice problems (neurogenic)				
Speech problems (neurogenic)				
Stroke				
Head trauma				
Tuberculosis infections (TBI)				
Multiple sclerosis (MS)				
Psyciatric disorders				
Drug and substance abuse				
Coma				

Cranial Nerve (CN) Examination (Must be "Normal" to include Research Participant)

Cranial Nerve	Normal	Abnormal	Description if Abnormal	Exclusion
I. Olfactory				
II. Optic				
III. Oculomotor				
IV. Trochlear				
V. Trigeminal				
VI. Abducens				
VII. Facial				
VIII. Vestibulocochlear				
IX. Glossopharyngeal				
X. Vagus				
XI. Accessory				
XII. Hypoglossal				

Compiled from:
1) Golper, L.A. (1992) as adapted from Gilroy, J. (1990). Basic Neurology. In J.W. Hall & H.G. Mueller. Audiologist's Desk Reference Vol 1 (pp 37 & 38) Singular Publishing: UK
2) Martin F.N. & Clark J.G. (2006) Introduction to Audiology Pearson / Allyn and Bacon: Boston. p422-423

SECTION E: AUDIOLOGICAL EXAMINATION

Completed by: _____

Exclusion Criteria (Must be "Normal" in Exclusion column to include Research Participant)

Otoscopic examination	Normal	Abnormal	Description if Abnormal	Exclusion
Condition of ear canal - Left				
Condition of ear canal - Right				
Condition of tympanic membrane - Left				
Condition of tympanic membrane - Right				

Acoustic Immittance:	RIGHT	LEFT	Exclusion
Tympanogram			
Middle ear pressure			
Static compliance			
Ear canal volume			

OAE:

Indicate which frequencies failed	RIGHT	LEFT	Exclusion
6000 Hz			
5000 Hz			
4000 Hz			
3000 Hz			
2000 Hz			

Pure Tone Audiogram:

Left ear pure tone air conduction		Right ear pure tone air conduction	
250 Hz	dB	**250 Hz**	dB
500 Hz	dB	**500 Hz**	dB
1000 Hz	dB	**1000 Hz**	dB
2000 Hz	dB	**2000 Hz**	dB
4000 Hz	dB	**4000 Hz**	dB
6000 Hz	dB	**6000 Hz**	dB
8000 Hz	dB	**8000 Hz**	dB

Are all the values 0 - 25 dB or better	Yes	No	All the values need to be 25 dB or less to include the Research Participant

Compiled from:
1) Jerger J. & Jerger S. Measurement of hearing in adults. In M.M. Paparella. & D.A. Shumrick (eds). Otolaryngology (2nd ed). Philadelphia: W.B. Saunders, 1908, p 1226. In Audiologist's Desk Reference Vol 1 (pp 104) Singular Publishing: UK
2) Stach B.A. (1998) Clinical Audiology: An Introduction. Singular Publishing: San Diego. London. p 200

SECTION F: OVERALL ELEGIBILITY FOR THE STUDY

Completed by: _____

Inclusion Criteria (Must be" YES" to include Research Participant)

Age of participants from 18-50 years?	**YES**	
	NO	
Research Participant has been informed of the nature and the aims of the study and who has given his/her written informed consent to participate?	**YES**	
	NO	
Participant is HIV positive?	**YES**	
	NO	
Section A: Biographical Information is inclusive?	**YES**	
	NO	
Section B: Medical History is inclusive?	**YES**	
	NO	
Section C: Magnetic Resonance Imaging (MRI) Compatibility?	**YES**	
	NO	
Section D: Clinical Examination of Auditory System is inclusive?	**YES**	
	NO	
Section E: Audiological Examination is inclusive?	**YES**	
	NO	
Section F: Overall Elegibility for the Study	**YES**	
	NO	
Inclusion for all Sections	**YES**	
	NO	
Scheduled fMRI	**Date**	
	Time	

APPENDIX D: POSTER

CLINICAL STUDY

Are you in the military service or a dependant of such a person, or someone who attends the Infectious Disease Clinic, male or female, aged 18-50 years?

Are you willing to participate in a two (2) day clinical trial to evaluate the brain's function of hearing to establish normal values ?

A response from you does not imply any obligation to participate.

If you are willing to participate, or need further information, please contact:

Mrs Celesté Pretorius

TEL: 082 419 17 49 / celestevh@msn.com

The study will be conducted in conjunction with the participation of a medical doctor.

APPENDIX E: ETHICAL CLEARANCE

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Research Ethics Committee

3 November 2010

Dear Dr Soer,

Project:	**The response of the central auditory nervous system to sound in normal hearing adults with and without HIV/AIDS: an fMRI study**
Researcher:	**C Pretorius**
Supervisor:	**Dr M Soer**
Department:	**Communication Pathology**
Reference number:	**21080870**

Thank you for your response to the Committee's letter of 12 October 2010.

I have pleasure in informing you that the Research Ethics Committee formally **approved** the above study at an *ad hoc* meeting held on 2 November 2010. Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should your actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

The Committee requests you to convey this approval to the researcher.

We wish you success with the project.

Sincerely

Prof. John Sharp
Chair: Research Ethics Committee
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: john.sharp@up.ac.za

1MH/302/6

Tel: 012 314 0487
Facsimile: 012 314 0013
Enquiries: Lt Col MK Baker

1 Military Hospital
Private Bag X1026
Thaba Tshwane
0143
5 October 2010

CLINICAL TRIAL APPROVAL PROTOCOL TITLE: "THE RESPONSE OF THE CENTRAL ADITORY NERVOUS SYSTEM TO SOUND IN NORMAL HEARING ADULTS WITH AND WITHOUT HIV/AIDS: AN FMRI STUDY"

1. The 1 Military Hospital Research Ethics Committee (1MHREC), comprised of the following members, and adhering to GCP/ICH and SA Clinical Trial guidelines, evaluated the above-mentioned protocol and additional documents:

a. Lt Col M. Baker: Neurologist, male, chairman 1MHREC.
b. Lt Col C.S.J. Duvenage: Specialist Physisian, female, member 1MHREC.
c. Lt Col. D. Mahapa: Dermatologist, female, member 1MHREC
d. Ms C. Jackson: Layperson, independent of the organization, female, member 1 MHREC.
e. Adv. T. J. Marè: Advocate, independent of the organization, male, member 1 MHREC.

2. The following study protocol was evaluated: **"The response of the central aditory nervous system to sound in normal hearing adults with and without HIV/AIDS: An fMRI study".**

3. The recommendations are: The study was approved on 4 October 2010. The principal investigator will be Mrs. C. Pretorius. Dr L.M. Hofmeyr will be the internal supervisor. Report backs are to be made to the 1MHREC six monthly, in the event of any serious adverse events and on completion or termination of the study.

M.K Baker

(M.K BAKER)
CHAIRMAN 1 MILITARY HOSPITAL RESEARCH ETHICS COMMITTEE: LT COL

DIST

For Action

Lt Col L.M. Hofmeyr

APPENDIX F: LETTER RE. LANGUAGE EDITING

ELSIE C NAUDÉ Spraakterapie/Speech Therapy
DPhil Kommunikasiepatologie/ Communication Pathology

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Sedgefield
6573

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ST0004871
Praktyk nr/ Practice no:
082 000 8202559

E-pos/E-mail: elsienaude@gmail.com
Tel/fax: (044) 343 3018
Sel/Cell: 082 825 6588

2017- 06-25

This is to affirm that the document

The response of the central auditory system to sound in normal hearing adults with and without HIV/AIDS: An fMRI study

CELESTÉ PRETORIUS

U10457900

was edited by me with regard to language and style.

Elsie Naudé

APPENDIX G: STATISTICAL AMENDMENTS

In Appendix G the statistical tests were done for the control of the normal distribution of groups, racial distributions and age disparity.

1. The assumption of a normal distribution

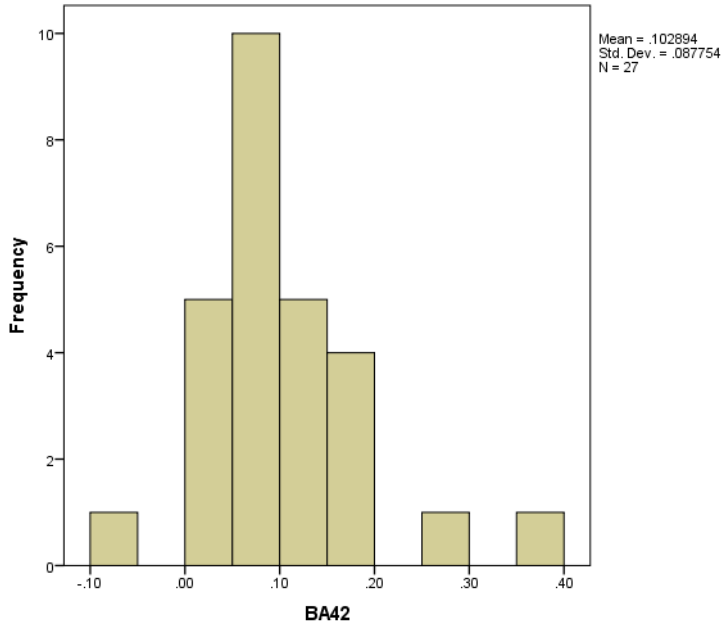
The assumption underlying the ANOVA F-test is not that the sample has a normal distribution, but rather that the error terms have a normal distribution. The error terms are estimated by the residuals:

Residual = observed value - predicted value.

The models for BA42 and BA44 were fitted and the residuals were tested for normality using the Kolmogorov-Smirnov one-sample test and by inspecting the histogram, while bearing in mind that the sample is small. (n=24).

BA42

Histogram of the residuals for BA42



The histogram does somewhat resembles a bell shape.

Kolmogorov-Smirnov test

The null hypothesis is because the distribution is normal.

One-Sample Kolmogorov-Smirnov Test

		Residual for BA42
N		27
Normal Parameters[a,b]	Mean	.0000
	Std. Deviation	.07266
Most Extreme Differences	Absolute	.150
	Positive	.150
	Negative	-.107
Test Statistic		.150
Asymp. Sig. (2-tailed)		.124[c]

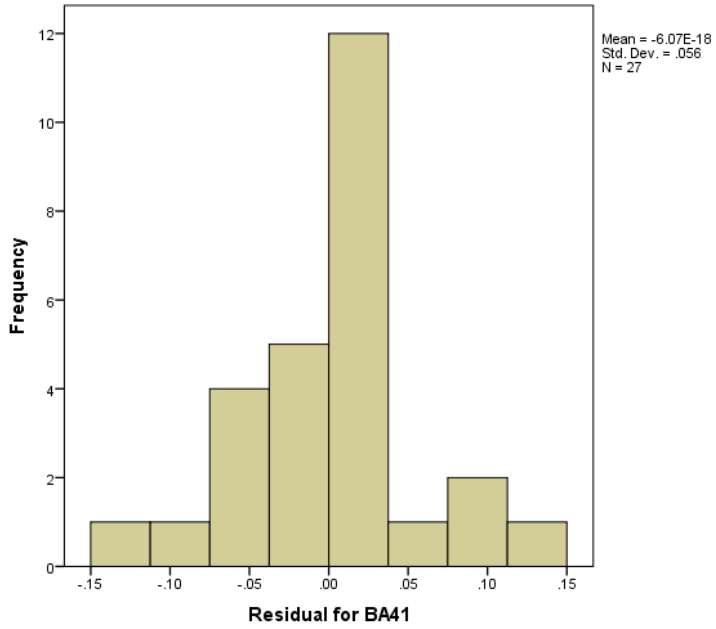
a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

The p-value is larger than 0.05, therefore the null hypothesis is **not rejected.**

BA41



Histogram of the residuals of BA41

Kolmogorov-Smirnov test

One-Sample Kolmogorov-Smirnov Test

		Residual for BA41
N		27
Normal Parameters[a,b]	Mean	.0000
	Std. Deviation	.05577
Most Extreme Differences	Absolute	.138
	Positive	.138
	Negative	-.096
Test Statistic		.138
Asymp. Sig. (2-tailed)		.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

The p-value is 0.200 which is larger than 0.05. Thus normality cannot be rejected.

2. The problem of imbalance in the racial distributions

The distributions are as follows:

GROUP * Race Crosstabulation

Count

		Race		Total
		Black	Caucasian	
GROUP	HIV	12	0	12
	Control	7	8	15
Total		19	8	27

None of the HIV/AIDS patients were Caucasian. An analysis was done using only the Black patients, 12 HIV patients and 7 Control.

Univariate Analysis of Variance

Between-Subjects Factors

		Value Label	N
Group	1	HIV	12
	2	Control	7
Gender	1	Male	7
	2	Female	12

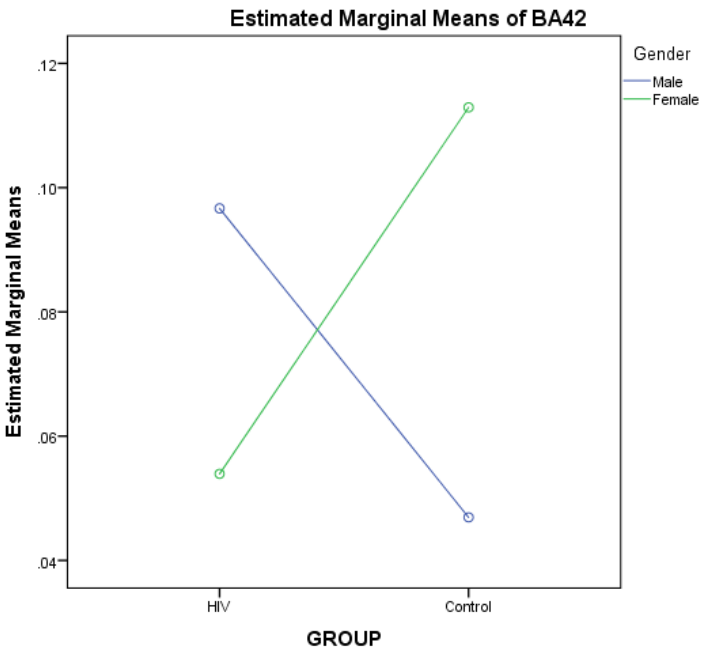
Tests of Between-Subjects Effects

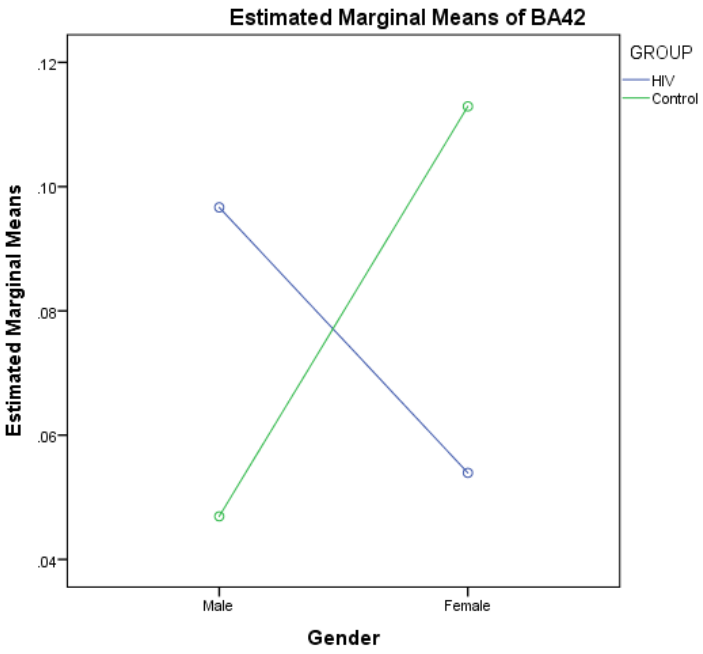
Dependent Variable: BA42

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.014[a]	3	.005	1.244	.329
Intercept	.101	1	.101	27.736	.000
GROUP	8.923E-5	1	8.923E-5	.025	.877
Gender	.001	1	.001	.156	.699
GROUP * Gender	.012	1	.012	3.400	.085
Error	.054	15	.004		
Total	.173	19			
Corrected Total	.068	18			

a. R Squared = .199 (Adjusted R Squared = .039)

Profile Plots





BA41

Univariate Analysis of Variance

Between-Subjects Factors

		Value Label	N
Group	1	HIV	12
	2	Control	7
Gender	1	Male	7
	2	Female	12

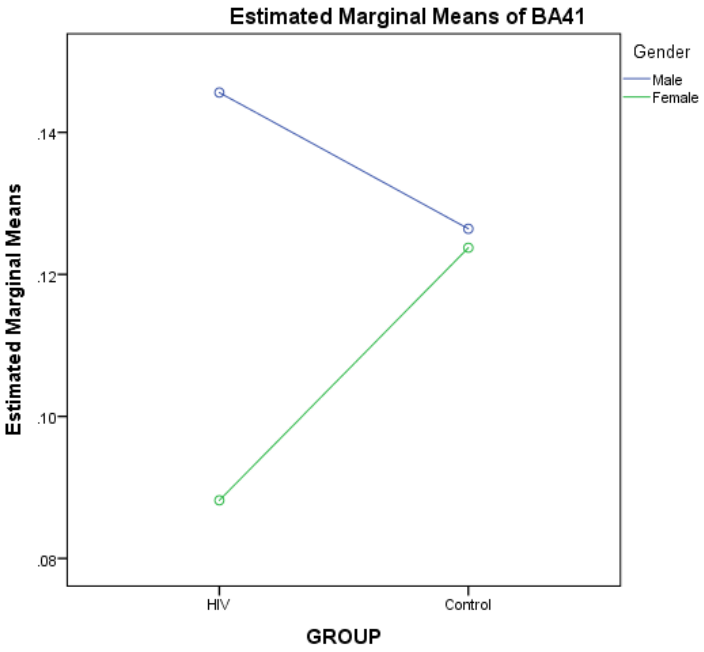
Tests of Between-Subjects Effects

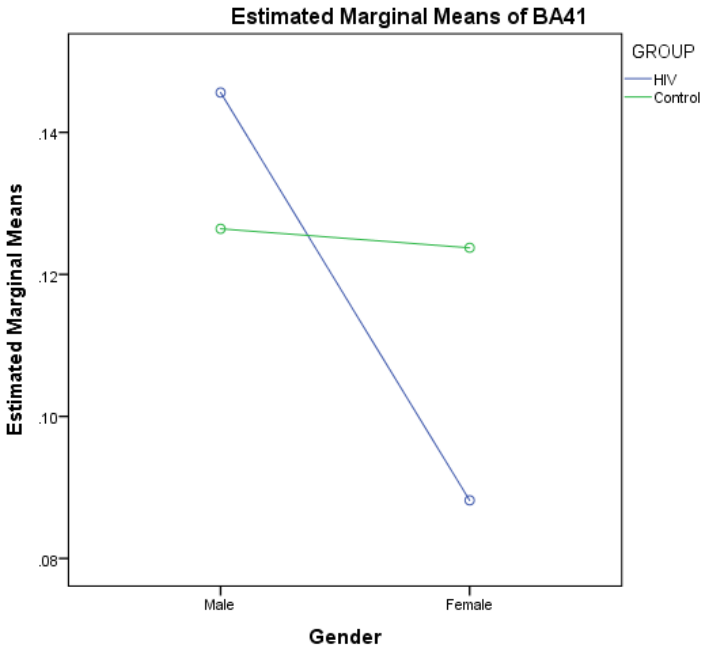
Dependent Variable: BA41

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.010[a]	3	.003	1.186	.349
Intercept	.244	1	.244	85.468	.000
GROUP	.000	1	.000	.098	.759
Gender	.004	1	.004	1.318	.269
GROUP * Gender	.003	1	.003	1.094	.312
Error	.043	15	.003		
Total	.299	19			
Corrected Total	.053	18			

a. R Squared = .192 (Adjusted R Squared = .030)

Profile Plots





The conclusions remain the same, whether the Caucasian participants are included or not.

3. Age disparity

The means are as follows:

Descriptive Statistics

GROUP		N	Minimum	Maximum	Mean	Std. Deviation
HIV	Age_years	12	23	43	32.83	6.492
	Valid N (listwise)	12				
Control	Age_years	15	21	36	25.67	5.602
	Valid N (listwise)	15				

To adjust for the difference in mean age between the HIV and Control groups, Age was added in the ANOVA as a Covariate (i.e. the means are adjusted for age).

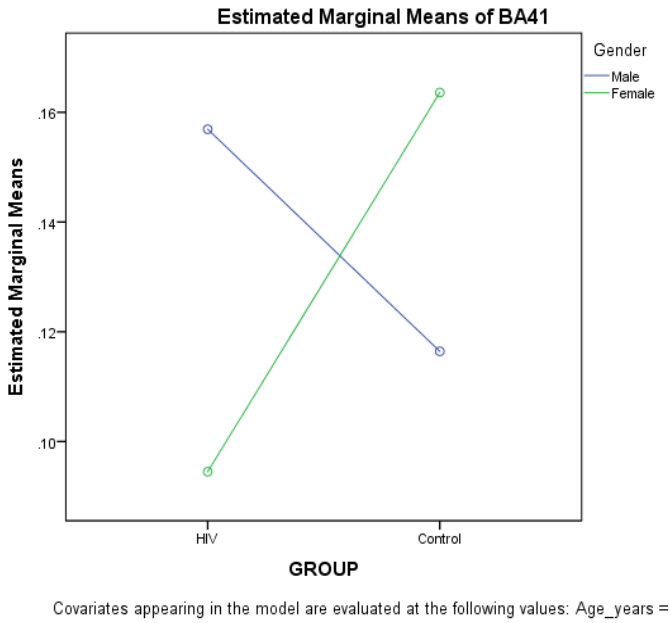
BA41

Univariate Analysis of Variance

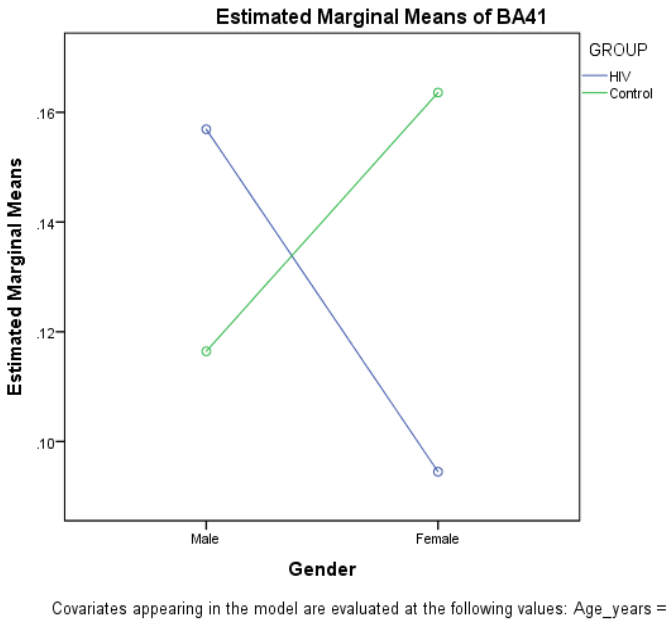
Between-Subjects Factors			
		Value Label	N
Group	1	HIV	12
	2	Control	15
Gender	1	Male	9
	2	Female	18

Tests of Between-Subjects Effects					
Dependent Variable: BA41					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.035[a]	4	.009	2.460	.075
Intercept	.036	1	.036	10.114	.004
Age_years	.004	1	.004	1.011	.326
GROUP	.001	1	.001	.250	.622
Gender	.000	1	.000	.097	.758
GROUP * Gender	.018	1	.018	5.020	.035
Error	.077	22	.004		
Total	.592	27			
Corrected Total	.112	26			
a. R Squared = .309 (Adjusted R Squared = .183)					

Profile Plots



Covariates appearing in the model are evaluated at the following values: Age_years = 28.85



Covariates appearing in the model are evaluated at the following values: Age_years = 28.85

The conclusions are the same with and without age.

BA42

Univariate Analysis of Variance

Between-Subjects Factors

		Value Label	N
Group	1	HIV	12
	2	Control	15
Gender	1	Male	9
	2	Female	18

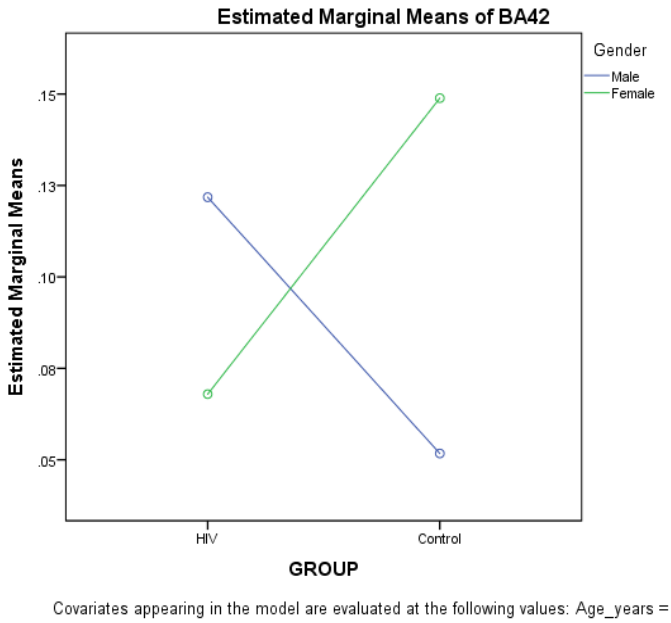
Tests of Between-Subjects Effects

Dependent Variable: BA42

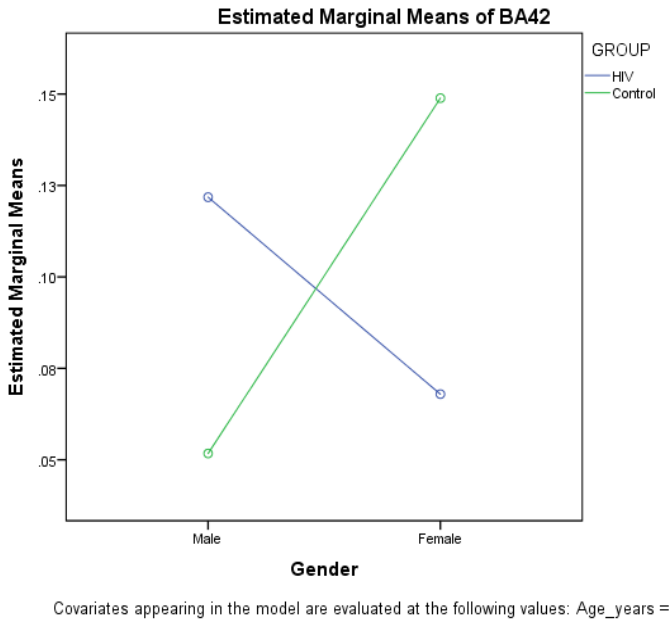
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.080[a]	4	.020	3.698	.019
Intercept	.050	1	.050	9.180	.006
Age_years	.018	1	.018	3.226	.086
GROUP	.000	1	.000	.023	.880
Gender	.003	1	.003	.504	.485
GROUP * Gender	.033	1	.033	6.152	.021
Error	.120	22	.005		
Total	.486	27			
Corrected Total	.200	26			

a. R Squared = .402 (Adjusted R Squared = .293)

Profile Plots



Covariates appearing in the model are evaluated at the following values: Age_years = 28.85



Covariates appearing in the model are evaluated at the following values: Age_years = 28.85

The conclusions are the same with and without age.