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Weinberg, Ian Robert

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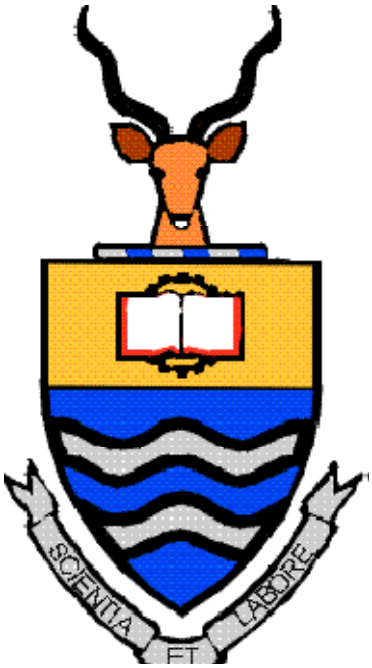
AMYGDALOID AND HIPPOCAMPAL VOLUMETRIC VARIATIONS AND THE EFFECTS OF THE FEELINGS OF HOPELESSNESS AND HELPLESSNESS



Ian Robert WEINBERG

Student number: 7349595

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Masters of Science in Medicine

SCHOOL OF ANATOMICAL SCIENCES

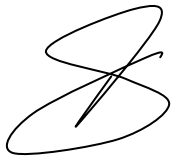
UNIVERSITY OF THE WITWATERSRAND

JOHANNESBURG

October, 2024

DECLARATION

I, Ian Robert Weinberg, declare that this Dissertation is myown, unaided work. It is being submitted for the Degree of Masters of Science in Medicine at theUniversity of the Witwatersrand, Johannesburg. It has not been submitted before forany degree or examination at any other University.



Ian WEINBERG

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ABSTRACT

Volumetry was performed on the basis pontis, amygdaloid body, and hippocampus proper in 700 normal MRI scans. The volume of the basis pontis was found to remain relatively constant across all age groups until the eighth decade. The consistency of volume of the basis pontis was used to derive volume ratios of the amygdaloid body and hippocampus proper. The volumes of the amygdaloid body and hippocampus proper remained relatively constant until the end of the sixth decade after which atrophy became significant. The volume ratio of the right hippocampus was greater than the left over all age groups. These analyses provided a baseline from which comparisons to clinical cases could be made. Volumetry of the same three structures was performed on a cohort of 80 clinical MRI scans and correlated with scores of hopelessness and helplessness derived from self-reporting questionnaires. A correlation was noted of hippocampal proper atrophy and amygdaloid body atrophy in relation to helplessness, but with questionable significance. Further studies exploring these relationships are required to fully determine potential relationships and diagnostic significance between amygdaloid body and hippocampus proper atrophy and neurodegenerative diseases, such as Alzheimer's disease.

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CHAPTER ONE | INTRODUCTION

The configurations of neuroanatomical structures reflect more than the basic function of individual elements and their integrations. As a consequence of the active engagement of the nervous system with the internal and external environments, the volumes and shapes of cerebral structures in the adult brain may change over protracted periods of time. These changes are most marked in the structures where adult neurogenesis occurs. Neurogenesis is the process whereby a new neuronal cell is generated from a progenitor cell. Studies have revealed that the process of adult neurogenesis is most active in the hippocampus and amygdala, with the result that significant changes in volume may manifest in the course of a lifetime (Eriksson et al., 1998; Roeder et al., 2022). Additionally the process of senile atrophy contributes significantly to volume involution in most cerebral structures (Fujita et al., 2023).

The neuroanatomy of the central nervous system therefore chronicles these multiple influences, inclusive of both intrinsic neurophysiology as well as internal and external environmental triggers, that are reflected in the collective neuroanatomical configuration (Fujita et al., 2023). This study has been undertaken with two primary aims: 1) To document volume changes in the amygdaloid body and hippocampus proper over a broad range of ages in non-clinical cases; and 2) To investigate the possible association of defined mind states with known volume configurations predisposing individuals to the development and progression of Alzheimer's disease.

1.1 | Review of the anatomy and physiology of the hippocampus formation and the amygdaloid body

The hippocampus formation and the amygdaloid body represent two of several structures that occupy the medial temporal lobes of the left and right cerebral hemispheres. These structures account for much of the cognitive and emotional processing of the brain.

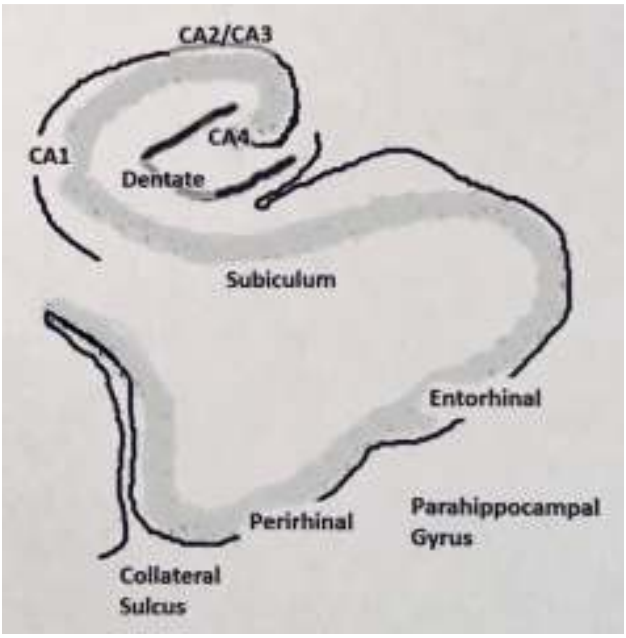
Effectively these structures integrate cognitive information with the physiology of emotion and episodic memory (Chauhan et al., 2021). Insights into the anatomy and physiology of these structures as well as their mutual interaction contributes to a greater understanding of the neuropathology emanating from these regions, thereby providing the basis for more effective intervention.

1.1.1 |The hippocampus proper

The hippocampal formation is comprised of the entorhinal cortex, subicular complex, cornu ammonis and the dentate gyrus. The hippocampus proper refers to the combination of the cornu ammonis and the dentate gyrus. The hippocampus proper forms an elongated structure occupying the infero-medial part of the temporal horn of the lateral ventricle, extending from the amygdaloid body anteriorly to the splenium of the corpus collosum posteriorly. The antero-posterior length of the hippocampus proper is approximately 5 cm and in the sagittal planecan be divided into the head or anterior segment, the body or intermediate segment and the tail or posterior segment (Dekeyzer et al., 2017). White matter fibres accumulate on the superior surface and become the alveus, that thickens into medial bundles as the fimbria and continues posteriorly as the fornix. In the coronal plane the hippocampus proper is divided into five parts based on the cytoarchitectural composition – CA1, CA2, CA3, CA4 and the dentate gyrus (Fig.1.1).

The cornu ammonis continues infero-medially, bordering the subiculum of the parahippocampal gyrus (Dekeyzer et al., 2017).The average volume of the human hippocampus proper is 2.9 cm^3 in the right hemisphere and 2.78 cm^3 in the left hemisphere (Honeycutt & Smith, 1995). Studies have consistently confirmed the increased volume of the right hippocampus proper relative to the left in individuals with normal cognitive function (Muller et al., 2005; Sarica et al., 2018).

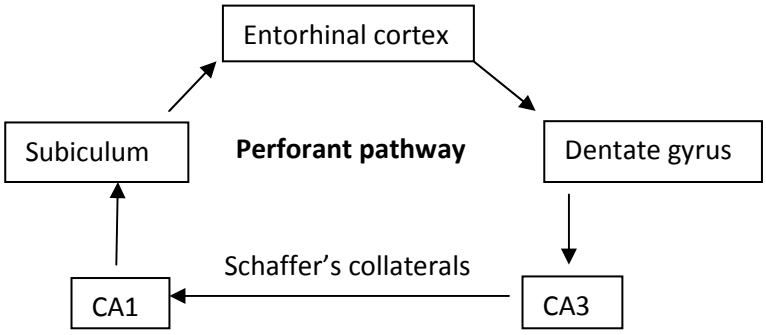
Figure 1.1: *Coronal view of a section through the hippocampal formation showing the five components of the hippocampus proper, as well as the subicular complex and entorhinal cortex*



Cytoarchitecturally, the dentate gyrus is noted to have three layers: 1) A superficial molecular layer; 2) the granular layer; and 3) the deeper polymorphic layer. The convexity of the folded dentate gyrus is directed towards the molecular layer while its concavity is directed towards the cornu ammonis. The dentate gyrus conveys most of the hippocampal afferent connections. The dendrites of granular neurons receive connections from neurons of the entorhinal cortex *via* the relevant segment of the perforant pathway. Axons of the granular cells synapse *via* mossy fibres with the apical dendrites of the pyramidal cells present in CA3 of the cornu ammonis (Chauhan et al., 2021). Some CA3 pyramidal axons synapse *via* collaterals with pyramidal cells in CA1. These collaterals are referred to as Schaffer's collaterals. The complete perforant pathway describes a circuit incorporating the entorhinal cortex, dentate gyrus, CA3, CA1 and subiculum. This pathway plays an important role in

short term memory, episodic memory, pattern recognition, the encoding of memory and the retrieval of information (Fig. 1.2).

Figure 1.2: *Diagrammatic representation of the perforant pathway*

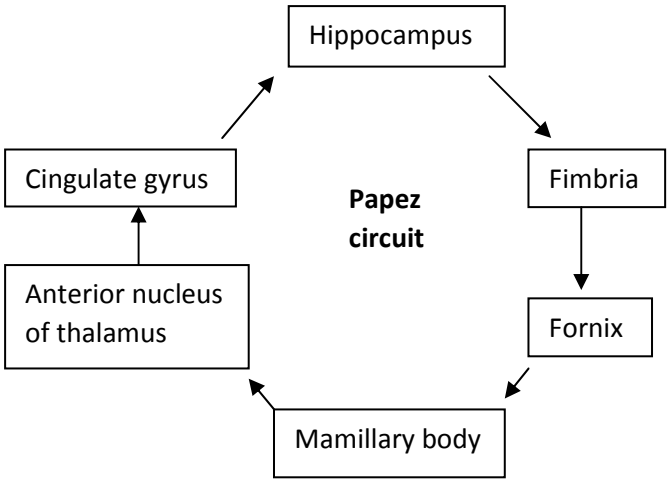


This pathway is also noteworthy for the severe degeneration seen in Alzheimer's disease (Anand & Dhikav, 2012). The pyramidal cells of the stratum pyramidalis form the principle cellular component of the cornu ammonis. The pyramidal cells have a base directed laterally (towards the alveus) and an apex directed medially (towards the dentate gyrus). Functionally, the pyramidal cells are excitatory and the axons arising from the base of the pyramidal cells of CA3 form the alveus. Dendrites arising from the base of the pyramidal cells give rise to the stratum oriens which also contains inhibitory basket cells. Basal dendrites in the stratum oriens receive commissural fibres from identical regions in the contralateral hippocampus (Chauhan et al., 2021). The CA1 segment comprises the main efferent region of the hippocampus proper (Yang & Wang, 2017). Axons from pyramidal cells in the dorsal CA1 project to the subiculum and entorhinal cortex as the initial segment of the perforant pathway while the ventral CA1 projects to the medial pre-frontal cortex, the nucleus accumbens and the amygdaloid body. The ventral hippocampus is responsible for

affective/emotional functions, while the dorsal hippocampus is primarily responsible for episodic memory and spatial navigation.

Emotional integration with memory is postulated to occur in the Papez circuit (Chauhan et al., 2021). The Papez circuit includes the: hippocampus proper, fornix, mamillary bodies, anterior nucleus of the thalamus and the cingulate cortex (Fig. 1.3).

Figure 1.3: *The Papez circuit*



The primary functions of the hippocampal formation, including the hippocampus proper, relate to: short-term or working memory; the conversion of short-term memory into more sustained memory through the process of long term potentiation (LTP); declarative memory; navigational memory; and relational memory. Declarative memory comprises episodic memory that relates to personal experience and semantic memory that relates to general factual details (Duff et al., 2020). Navigational memory or spatial mapping relates to the function of place cells in the hippocampus (Konkel & Cohen, 2009). Relational memory relates to cognitive mapping and integration as well as cognitive and emotional integration (Konkel & Cohen, 2009). Relational memory supports social interaction and by its nature reflects hippocampal cognitive integration with emotional memory (Rubin et al., 2014).

1.1.2 | The amygdaloid body

The amygdaloid body occupies a position antero-superior to the head of the hippocampus and is defined as those nuclei forming a distinct anatomical structure in the temporal lobe (Imam et al., 2022). The structure consists of both pallial and subpallial portions, leading to the development of the amygdaloid body and the extended amygdala respectively (Imam et al., 2022). It has been shown that the amygdaloid body of humans has an average volume of 1.05 cm^3 in the right hemisphere and 1.14 cm^3 in the left hemisphere (Convit et al., 1999).The amygdaloid body is divided into three identifiable groups of nuclei: 1) The baso-lateral group that contains the lateral nucleus, the basal nucleus and the baso-medial nucleus. This group can be further divided into the anterior part and the posterior part ; 2) The cortical-like group that comprises the nuclei of the lateral olfactory tract and the cortical nuclei; and 3) The centro-medial group that includes the central and medial nuclei (Yang & Wang, 2017).

Anatomically the lateral nucleus receives most of the afferent connections. The lateral nucleus in turn projects to multiple amygdaloid nuclei. There are two major efferent pathways from the amygdala:1) the dorsal route projecting *via* the stria terminalis to the septal area, bed nuclei of the stria terminalis, and hypothalamus; and 2) the ventral route projecting *via* the amygdalofugal pathway and terminating in the septal area, hypothalamus and the medial dorsal thalamic nucleus. The amygdaloid body processes information that is conveyed between the prefrontal cortex, the temporal association cortex and the hypothalamus (AbuHasan et al., 2024). There are also connections from the amygdala to the ventral pallidum and ventral striatum that are relayed back to the cortex *via* the dorsomedial nucleus of the thalamus. An important circuit has been proposed to support the amygdaloid function allowing the ability to infer the intention of others from their language, gaze and gestures (AbuHasan et al., 2024). This circuit incorporates: the basolateral amygdala, the

orbitofrontal and anterior temporal cortices and the dorsomedial nucleus of the thalamus (AbuHasan et al., 2024). The dorsomedial nucleus of the thalamus relays information back to the orbitofrontal cortex *via* the frontothalamic tract (AbuHasan et al., 2024).

The primary functions of the amygdaloid body include fear conditioning and emotional memory and it is also involved in regulating anxiety, aggression and social conditioning (Langevin, 2012; Shin &Liberzon, 2009; Gorman et al., 2000; AbuHasan et al., 2024). The baso-lateral nucleus plays an integral role in anxiety processing, while fear conditioning appears to be a primary function of the lateral nucleus (Yang & Wang, 2017). In evolutionary terms the amygdaloid complex is central to survival, as demonstrated by its 'fight or flight' function. The basolateral amygdaloid nuclei are central to enabling the fear function and are known to contain an abundance of beta 2 adrenergic receptors (Nikolenko et al., 2020). As the amygdaloid body is stimulated by the very same chemicals that it causes to be secreted (adrenaline/noradrenaline and cortisol), the survival function of the amygdala becomes amplified (Davis, 1992; Kukolja et al., 2008; Nikolenko et al., 2020).

1.2 | The amygdaloid body and hippocampus proper interactions

Both the amygdaloid body and hippocampus proper exhibit significant volumetric variation due to the fact that both structures incorporate stem cells giving rise to progenitor neuronal cells and mature neurons throughout life (Eriksson et al., 1998; Roeder et al., 2022). This process of neurogenesis inclusive of adult neurogenesis and associated neuroplasticity, contributes to measurable changes in terms of relative volume variations throughout life reflecting psycho-social influences mediated for the most part *via* the hypothalamic-pituitary-adrenal (HPA) axis. Free of extreme influences, the amygdaloid body and hippocampus proper tend to maintain a relative constancy of volume until just prior to the onset of the sixth

decade, following which the rate of involution exceeds that of the cerebral cortex (Fujita et al., 2023).

Important anatomical connections and physiological processes have been identified as existing between the amygdaloid body and the hippocampus proper and these mediate significant influences. Anatomically, efferent fibres originating in the baso-lateral nucleus of the amygdaloid body connect to the ventral hippocampus with additional connections existing between the cortical amygdaloid nuclei and the hippocampus (CA3, CA1 and ventral subiculum) (Majak & Pitkanen, 2003; Meissner et al., 2022).

Physiologically, much of the interactions between these structures are based on the existence of an abundance of cortisol receptors in both the amygdaloid body and hippocampus proper (Tottenham & Sheridan, 2010). Consequently both structures become major targets of an activated HPA axis. Increased cortisol secretion resulting from the triggering of the HPA axis by an activated amygdaloid body results in both further stimulation of the amygdaloid body as well as suppression of hippocampal function (McEwen, 1998). Prolonged cortisol dysregulation of the hippocampus results in the atrophy of cells in the CA3 region (McEwen et al., 2016).

The activated HPA axis also results in raised levels of adrenalin that has been shown to upregulate the secretion of pro-inflammatory cytokines (Vale, 2005). Evidence indicates that raised levels of pro-inflammatory cytokines contribute to hippocampal atrophy (Sudheimer et al., 2014). Raised levels of pro-inflammatory cytokines have been shown to mediate inflammation in the presence of raised cortisol levels (cortisol resistance) by impairing the function of glucocorticoid receptors (Pace et al., 2007).

In this way, the interrelatedness of the physiology of the amygdaloid body and hippocampus proper supports the convergence of cognitive and emotional function and mediates the influences upon relevant volumetry.

CHAPTER 2 | LITERATURE REVIEW

2.1 | Review of psycho-social influences on volumetric analyses

Changes in the volumes of the amygdaloid body and hippocampus proper that are associated with psycho-social events that are mediated by the physiology of the HPA axis have been identified as commencing at an early age (Tottenham & Sheridan, 2010). Volumetric studies of children reared in adverse conditions associated with nurture deprivation reveal significant hypertrophy of the amygdaloid body when compared with control groups. While the hippocampus was also noted to be smaller in the studied cohort as compared to those in the control group, this was not statistically significant in this age group (Gilhooly et al., 2010).

Adverse nurture conditions become embedded in the biology of the amygdaloid body and hippocampus proper, manifesting in later life with changes in physiology related to the HPA axis and changes in volumes of the involved structures. Increased levels of nurture adversity predispose individuals to the adult development of anxiety and depression and a decrease in hippocampal volumes (Tottenham & Sheridan, 2010).

Earlier studies have shown that atrophy of the hippocampus proper was associated with stress or 'allostatic load' (McEwen, 1998). Atrophy of the hippocampus proper has also been shown to be associated with post-traumatic stress disorder (PTSD) (Shin & Liberzon, 2009). In a study of PTSD, significant atrophy of the hippocampus proper was observed, but without associated changes in the volume of the amygdaloid body (Bremner et al., 1997). Significant atrophy of the hippocampus proper has also been observed in association with major depression (Sheline et al., 1996). Late onset depression or multiple episodes of depression was associated with lower volumes of the hippocampus proper (Oyarce et al., 2020).

In a recent study both amygdaloid body and hippocampus proper atrophy was observed in adults suffering from major depression and who had experienced childhood trauma (Weissman et al., 2020). While increased amygdaloid body volumes have been noted in association with depression, a significant percentage of those living with chronic depression progress to amygdaloid body atrophy (Tottenham & Sheridan, 2010). Increased left amygdaloid body volume has been observed in association with chronic anxiety while the presence of this co-morbid chronic anxiety in the context of chronic depression appears to diminish the degree of hippocampus proper volume loss due to the chronic depression (Oyarce et al, 2020).

Collating these findings one can discern the following: 1) the chronicity of depression appears to correlate with increasing atrophy of the hippocampus proper; and 2) in the presence of significant anxiety, amygdaloid body hypertrophy exists. This is discernable from early childhood, where adverse nurture deprivation appears to be the precipitating cause. The existence of the co-morbidity of anxiety with depression appears to limit the degree of hippocampus proper atrophy. In this situation amygdaloid body hypertrophy has been noted to exist in association with chronic depression; however a significant percentage of amygdaloid body hypertrophy progresses to atrophy in the presence of chronic depression giving rise to a state of combined amygdala and hippocampal atrophy.

2.2 | The states of hopelessness and helplessness

Henkel et al stated, “According to cognitive-behavioural theories, helplessness and hopelessness are rather considered to be potential aetiological factors as antecedent causes for the onset or maintenance of depression. In this context, helplessness and hopelessness are considered as *trait* factors which enhance the vulnerability of a person to react to certain environmental stimuli with depression. In these concepts, helplessness and hopelessness exist

independently from depressive episodes as individual attitudes representing vulnerability and risk factors" (Henkel et al., 2002 pp. 240).

Therefore the interpretation of hopelessness and helplessness is that together they may precipitate clinical depression, sustain clinical depression, and determine the effectiveness of intervention. Inthis way hopelessness and helplessness may be regarded as the states that may progress to the development of clinical depression. The state of hopelessness may be further defined as a cognitive vulnerability that evolves into depression when exposed to a stressful life event (Haeffel et al., 2017).

Helplessness is more commonly referred to as learned helplessness. Learned helplessness usually ensues from an inescapable stressful life event including an adverse nurture experience. Learned helplessness results in a mind state of resignation and futility when faced with recurring adversity and the subjective belief in the permanence of the adversity (Maier &Seligman, 2016).Of significance in the context of learned helplessness is the finding that learned helplessness gives rise to atrophy of the hippocampus proper. Cell proliferation, or the rate of adult hippocampal neurogenesis, was found to be decreased in response to inescapable stress (Malberg & Duman, 2003).

2.3 | Volume configurations of the amygdaloid body and hippocampus proper in regard to Alzheimer's disease

Alzheimer's disease was first reported by Alois Alzheimer in 1907. The pathogenicity of the condition is characterized by the accumulation of ß-amyloid (ß-A) and the over expression of phosphorylated Tau protein in the brain. In Alzheimer's disease the ß-A peptides aggregate to form insoluble fibrils in plaques and blood vessels while the phosphorylated Tau forms into neurofibrillary tangles. Both of these components are neurotoxic. Patients with Alzheimer's disease present with cognitive and behavioural

impairment progressing to dementia together with the psychological symptoms of anxiety, depression and aggression (Takahashi et al., 2023).

Atrophy of the amygdaloid body, usually in association with chronic depression, becomes a prominent feature in the early phases of Alzheimer's disease. Additionally, evidence indicates that the amygdaloid body may be a source of the prion protein ß-A as well as Tau proteins (Nikolenko et al., 2020). In regard to hippocampal volumes, more recent studies have highlighted the importance of the volume discrepancies between the right and left hippocampus proper in the context of Alzheimer's disease. Individuals with normal cognitive function exhibit a significantly larger right sided hippocampus proper than left; however decreasing cognitive function is associated with an increasing volume difference between right and left hippocampi proper (right greater than left) with the greatest difference noted in established Alzheimer's disease (Sarica et al., 2018). The decreased volume was reflected in both the hippocampus proper as a whole as well as in eight of twelve subfields (Sarica et al., 2018).The temporal progression of hippocampal atrophy may occur over a protracted period of time. Atrophy of the hippocampus proper has been observed to pre-empt the development of Alzheimer's disease by up to 20 years (Gosche et al., 2002).

It is apparent that depression and anxiety contribute to hippocampal atrophy, as depression is both precipitated and sustained by hopelessness and helplessness, indicating that these states together may be regarded as predisposing an individual to atrophy of the hippocampus proper (McEwen, 1998; Oyarce et al., 2020). The probable medium of influence of these emotional states is the generation of chronic inflammation following on from the finding that depression, incorporating the elements of hopelessness and learned helplessness, both stimulates the secretion of pro-inflammatory cytokines and a milieu of chronic inflammation, which in itself further sustains the causative emotional states (Kiecolt-Glaser et al., 2015). This is further compounded by the increased secretion of pro-

inflammatory cytokines resulting from states of anxiety and the raised levels of adrenalin arising from the activated HPA axis (Vale, 2005).

Chronic inflammatory states have also been shown to negatively affect the processes of neuroplasticity in the hippocampus proper (Khairova et al., 2009). While physiological levels of pro-inflammatory cytokines promote hippocampal function and learning, chronically raised levels suppress this function and contribute to hippocampal atrophy and possible Alzheimer's disease (Bourgognon & Cavanagh, 2020).

The pathophysiological dynamic that emerges is one in which chronic depression underpinned by states of hopelessness and learned helplessness, complicated by chronic states of anxiety, predisposes individuals to chronic inflammation. The state of chronic inflammation negatively affects neurogenesis as well as neuroplasticity in the hippocampus proper giving rise to hippocampal atrophy. Chronic inflammation is noted to be a consistent feature of Alzheimer's disease.

2.4| Hippocampal atrophy as a risk variable predisposing to Alzheimer's disease

A review of multiple studies reveals that excessive hippocampal proper atrophy relative to normal mean volumes for age is the most consistent predictor of the progression to Alzheimer's disease (Henneman, 2009). The pathological progression to hippocampal atrophy is posulated to be due to a state of chronic inflammation precipitated by an excessively and chronically activated HPA axis. This pathological state is associated initially with amygdaloid body hypertrophy (Oyarce et al., 2020). It is suggested that the primary initiator of this process is the raised levels of cortisol interfacing with the abundance of cortisol receptors in both the amygdaloid body and hippocampus proper (Tottenham & Sheridan, 2010).

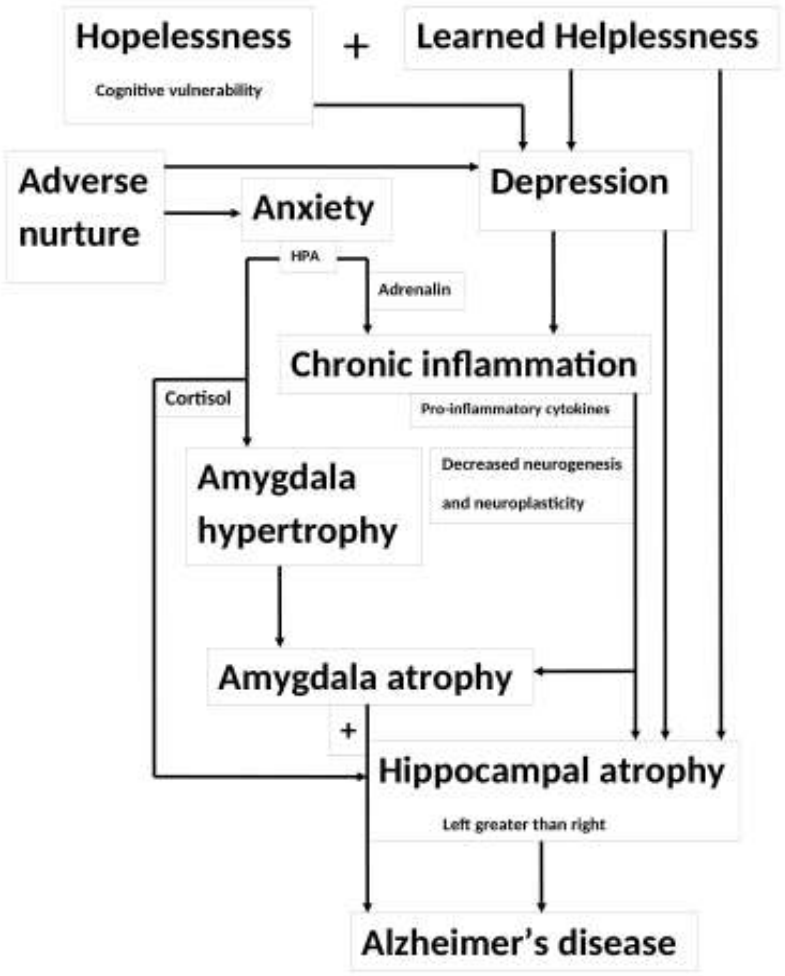
Raised cortisol activity tends to sustain amygdaloid stimulation in a positive feedback process while suppressing hippocampal function (McEwen et al., 2016; Nikolenko et al., 2020). Chronic cortisol stimulation of the hippocampus proper predisposes to hippocampal atrophy (McEwen et al., 2016; Oyarce et al., 2020). An additional aggravating factor in this regard is that the hippocampus in normal physiological conditions diminishes the cortisol stimulatory effect on the amygdaloid body. The suppression of hippocampal function and subsequent atrophy will result in excessive amygdaloid activity with increased cortisol production and further hippocampal suppression (Tottenham & Sheridan, 2010). The activated HPA axis also gives rise to raised levels of adrenalin (Sheng et al., 2021). Adrenalin stimulates the secretion of pro-inflammatory cytokines which have been shown to further predispose to hippocampal proper atrophy when chronically raised (Bourgognon & Cavanagh, 2020).

A connection exists between the ventro-medial prefrontal cortex and the amygdaloid body. In normal physiological conditions this connection mediates a suppressive effect upon the amygdaloid body (Tottenham & Sheridan, 2010); however in conditions of chronic anxiety and depression this suppressive connection becomes atrophied thus removing the inhibitory effect from the amygdaloid body. This process is further aggravated by the inhibitory effect of chronically raised levels of adrenalin on the ventro-medial prefrontal cortex (Arnsten, 2009).

The emotion of learned helplessness gives rise to atrophy of the hippocampus proper. Cell proliferation, the rate of adult hippocampal neurogenesis, was found to be decreased in response to inescapable stress, presumably as a consequence of the activated HPA axis-raised adrenalin-increased pro-inflammatory cytokine pathway (Malberg & Duman, 2003). Chronic learned helplessness is also associated with increased cortisol levels that may further contribute to hippocampal proper atrophy (Henkel et al., 2002).

It is postulated that the states of hopelessness and learned helplessness contribute to the development of Alzheimer's disease by virtue of the atrophy of the hippocampus proper precipitated by these states (Lindberg et al., 2012). Contributing to this progression is the finding that chronic anxiety independent of depression is associated with a higher incidence of Alzheimer's disease (Garcia et al., 2023). The multiple influences contributing to hippocampus proper atrophy and the predisposition to Alzheimer's disease are summarised in Figure 2.1.

Figure 2.1: *Influences contributing to hippocampal proper atrophy and the predisposition to Alzheimer's disease*



2.5 | Current theories in the aetiology of Alzheimer's disease

Alzheimer's disease is characterised by progressive atrophy of multiple cerebral structures diverging from normal rates of senescent atrophy (Blinkouskaya & Weickenmeir, 2021). Initial pathological atrophy commences in the hippocampi and is progressive. This appears to result from the neurotoxicity of accumulated and insoluble ß-A as well as Tau protein tangles (Kinney et al., 2018).

More recently attention has been directed at chronic inflammation as a possible third factor contributing to the pathological progression (Neuroinflammation Working Group, 2000). Chronic inflammation has been noted to be a ubiquitous accompaniment of Alzheimer's disease, both in the living individual and in post-mortem studies (Kinney et al., 2018). The chronic inflammatory process has however, been regarded as a secondary phenomenon precipitated and maintained by the accumulation of ß-A and Tau protein.

Studies have revealed compelling evidence in which the mediators of peripheral inflammation cross the blood-brain barrier and initiate states of chronic inflammation within cerebral tissue, often followed by the identification of ß-A development (Kinney et al., 2018). Evidence also indicates the possibility of peripheral organisms crossing the blood-brain barrier and initiating similar processes (Xie et al., 2022). Similarly, ß-A has been identified in cerebral tissue following blunt head injury and the ensuing inflammatory process (Neuroinflammation Working Group et al., 2000).

In the area of psycho-social influences the subject of oxytocin has increasing relevance in regard to Alzheimer's disease. Oxytocin, secreted by the posterior pituitary gland, is both a peptide hormone and neurotransmitter with widely distributed receptors in the hippocampal formation (Takahashi et al., 2023). Oxytocin has been shown to regulate anxiety and depression as well as cognitive and social functions. Social bonding increases oxytocin

secretion while social isolation diminishes its production. Raised levels of oxytocin decrease cortisol levels. Social isolation has been shown to increase the risk for progression to Alzheimer's disease by 51% (Takahashi et al., 2023). Social isolation is associated with consistently raised pro-inflammatory mediators which is proposed as one of the causal elements for the progression to Alzheimer's disease (Van Bogart et al., 2022). Social isolation has also been recognised as contributing to the increased cerebral atrophy of senescence (Lammer et al., 2023). Evidence indicates that the atrophy may be caused by the sensitization of microglia and chronic cerebral inflammation (Niraula et al., 2017). Emerging studies show a significant correlation between sensitized microglia and chronic inflammation in adulthood with childhood adversity (Chen et al., 2021).

The possibility exists therefore that neuropsychological states that have been shown to initiate and perpetuate states of chronic cerebral inflammation *de novo* may initiate and perpetuate the processes giving rise to the accumulation of ß-A fibrils and Tau protein, pathological cerebral atrophy, culminating in Alzheimer's disease.

2.6 Overview of the aims and methods of the study

The amygdaloid body and hippocampus proper are the focus of two different, but overlapping, areas of interest: 1) a psychiatric enquiry centred upon anxiety and depression; and 2) a neurological enquiry centred upon hippocampal atrophy and the possible progression to Alzheimer's disease. Many studies have established correlations between degrees of anxiety and depression with volumetric variations of the amygdaloid body and hippocampus proper (Sheline et al., 1996; Shin & Liberzon, 2009); however, relatively few studies have attempted to integrate the two areas of enquiry (Gosche et al., 2002). If indeed it can be shown thatin addition to volumetric correlations with emotional states there is also a progression to Alzheimer's disease, important new possibilities may emerge regarding earlier diagnosis and intervention of Alzheimer's disease.

This study was undertaken to evaluate the influences of the quantified emotional states of hopelessness and learned helplessness and their effects upon the volumes of the amygdaloid body and the hippocampus proper. The initial aim of the study was to quantify the volumes of the respective structures derived from a retrospective volumetric analysis of archived MRI scans. Once quantification was completed, a cohort of clinical subjects was evaluated in regard to correlations between the quantified mind states of hopelessness and learned helplessness with volumetric studies.

CHAPTER 3 | METHODS

3.1 | Participants

All radiological data included in the current study was extracted from the electronic records of a private radiological practice. Clinical privilege permitted unrestricted access to MRI scans. Ethics approval for this study was obtained from the Human Research Ethics Committee (Medical) of The University of the Witwatersrand, Johannesburg (clearance number M230110) (Appendix 1). The MRI scans utilized in the current study was comprised of two groups: a) Group 1, a large group of neurologically normal (non-clinical) whole brain scans; and b) Group 2, a smaller group of whole brain scans obtained from clinically relevant subjects.

3.1.1 | Group1 (Non clinical)

This group of 700 neurologically normal scans was randomly selected from our data repository and stratified into age groups 18-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80 and above (Table 3.1). Of the total of 700 scans, 448 were females (64%) and 252 were males (36%). Scans were excluded from this sample if gross anatomical pathology was notedon radiological examination. Following preliminary radiological examination, the scans were accessioned into the archive using the archived scan number and stored securely until further analysis. No personal details were included in the stored database. Individual consent was not required for individual scan analysis in this sample.

Table 3.1: *Group 1 (Non clinical) combined sex age distributionby cohort, mean age (in years) and standard deviation(SD). N = sample size*

Age cohort	N	Mean age, (SD)
18 - 19	16	18.69 (0.48)
20 - 29	70	24.81(2.91)
30 - 39	118	35.23 (2.71)
40 - 49	113	44.53 (2.96)
50 - 59	123	54.74 (2.85)
60 - 69	121	64.3 (2.74)
70 - 79	109	73.81 (2.96)
80 - 88	30	83.63 (2.19)

3.1.2 | Group 2 (Clinical)

This group consisted of a cohort of 80 scans of clinical subjects who had also completed self-reporting questionnaires. The subjects were stratified into age groups 18-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80 and above (Table 3.2). There were 55 females (69%) and 25 males (31%). The subjects were self-selected from patients who had been referred for MRI scans and who were invited to partake in the study. Participation was voluntary and without any financial incentive. Patients with cognitive dysfunction were excluded by the attending physicians. The subjects were presented with an information document that described the study and provided assurance of strict confidentiality. Additionally, free psychological counselling was made available in the event that the required self-reporting of the questionnaire resulted in any negative emotional consequences.

Table 3.2: *Group 2 (Clinical) combined sex age distribution by cohort, mean age (in years) and standard deviation (SD). N = sample size*

Age cohort	N	Mean age, (SD)
18 - 19	4	18.25 (0.5)
20 - 29	15	23.13 (2.2)
30 - 39	10	35.2 (3.22)
40 - 49	18	44.22 (3.23)
50 - 59	11	54.64 (3.26)
60 - 69	10	64.8 (2.1)
70 - 79	10	73.0 (2.91)
80 - 88	2	81.5 (0.71)

The subjects were presented with self-reporting questionnaires after providing written consent for participation. The two questionnaires utilized in this study were the Beck's Hopelessness Inventory (BHI) and the Learned Helplessness Scale (LHS) comprising 20 questions in each (Beck et al., 1974; Quinless & Nelson, 1988) (Appendices 2 and 3). The BHI provides quantification in regard to degrees of depression as well as pessimism. Affective, motivational and cognitive components were reflected in the score.

The hopelessness score for the BHI questionnaire ranges from 0 – 20 where 0 – 3 is minimal; 4 – 8 is mild; 9 – 14 is moderate: 15+ is severe. Earlier studies have validated the use of the LHS as a robust and reliable surveying instrument for evaluating learned helplessness (Quinless & Nelson, 1988) and demonstrated a correlation to the BHI score. The learned helplessness score on the LHS ranges from 20-80 with higher scores correlated with increased learned helplessness (Quinless & Nelson, 1988). The mean score of the LHS on

validation studies was 39.12 with a standard deviation of 6.32 (Quinless & Nelson, 1988). The MRI scans were analysed and stored together with the accompanying questionnaire data with the accession number used for identification. No personal details were included in the stored database.

3.2 | MRI image acquisition and processing

Scanning was performed at the DRS Radiological practice at Netcare Linksfield Hospital. We used manual segmentation procedures to isolate and reconstruct the volume of the regions of interest. Despite the time intensive nature of manual segmentation procedures, this method is recognized as the "gold standard" for MRI image analysis and is assessed as providing more accurate results when compared to existing automated approaches (Schoemaker et al., 2016). The regions of interest subjected to volumetry included the bilateral amygdaloid body and hippocampi proper as well as the volume of the basis pontis which was used as a reference volume. The basis pontis was selected as a reference volume based on its relative minimal involution and loss of volume when evaluated from the second decade through to the eighth decade (Raz et al., 2001). Relative changes in size of the hippocampus and amygdala were expressed as ratio's with respect to the basis pontis. All regions of interest (ROI's) were demarcated in axial, coronal and sagittal planesusing the accompanying ROI drawing and image analysis tools.Segmentation outlines were completed by IW with close reference to the published MRI and cytoarchitectural atlasof the human brain (Mai et al., 2015).

During a pilot study, a small subset of anterior-posterior ROI's were visually inspected by a second investigator (PRM)and checked for consistency with the anatomical landmarks.Strong congruence was observed between the published atlas and the ROI's identified by each observer, emphasising the accuracy and repeatability of this manual

approach.The choice of using a 1.5 Tesla MRI scanner rather than a 3.0 Tesla (or greater)waspragmatic based on availability but also theoretically guided as earlier studies have demonstratedless artefact originating from the base of skull when scanning using the 1.5 Tesla machine (Wardlaw et al., 2012). The loss of a higher resolution derived from a greater magnitude of magnetic field was more than compensated for by the diminished artefact, specifically in relation to the three structures analysed

3.3 | Volumetry of the basis pontis, amygdala and hippocampus in Group 1 (Non clinical)

The basis pontis volume was measured using Lambert's formula for an ovoid (Liu, et al., 2021). The required parameters were obtained from measurements in the sagittal and axial planes (Fig. 3.1). This method was found to be the most accurate in the volumetric assessment of an ovoid (Hsieh et al., 2009). The method was evaluated empirically in a volume displacement study and correlation with actual measurements prior to the onset of the study (Appendix 4).The amygdaloid body volume was measured using a similar method also employing Lambert's formula and obtaining the required parameters from measurements in the axial and coronal planes. The sagittal plane was employed to clarify the outer limits. In accordance with published anatomical descriptions, the superior extent of the amygdaloid body was identified as corresponding to a line connecting the most inferior point of the sylvian fissure with the most lateral part of the suprasellar (chiasmatic) cistern and, more posteriorly, the superior and lateral border of the optic tract. The inferior and lateral borders were determined by the boundary between the amygdaloid body and adjacent white matter. The medial border was determined by the boundary of the amygdaloid body and the suprasellar (chiasmatic) cistern. The most rostral extent was taken as the point where the

Figure 3.1:*A representative screenshot showing the computation of basis pontis volume in a de-identified subject.*

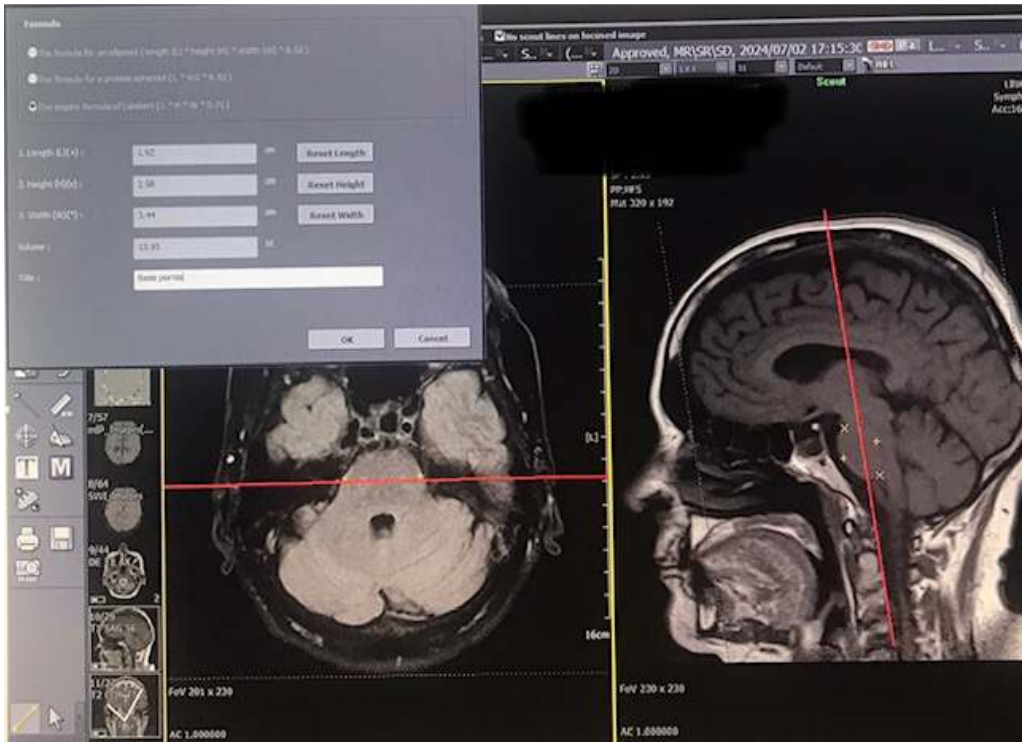
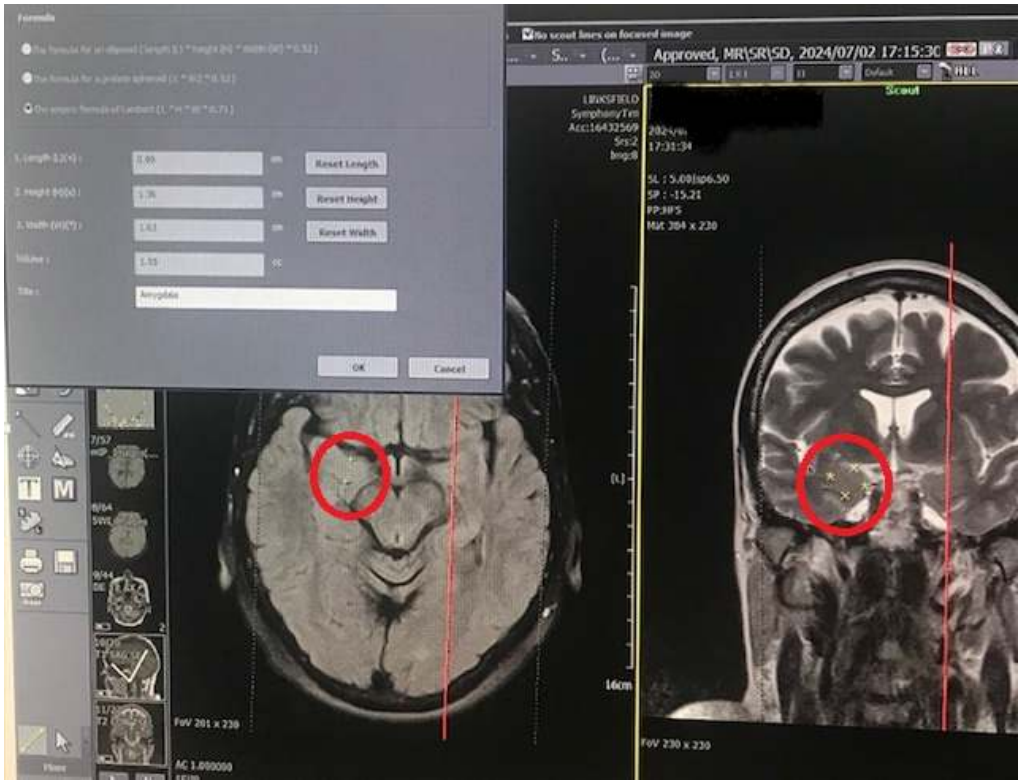


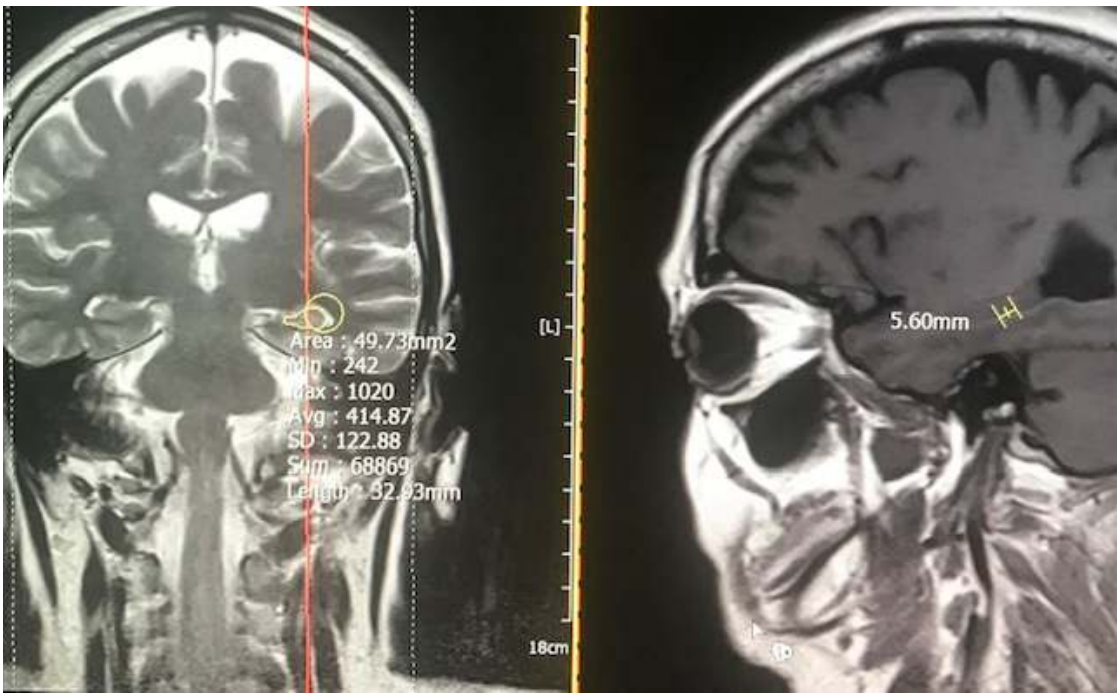
Figure 3.2: *A representative screenshot showing the anatomical location and computation of amygdaloid body volume in a de-identified subject. Note the screenshot is radiological orientation so the ROI (in RED) is pointing to the subjects right amygdala*



temporal and frontal lobes were no longer separate. The posterior boundary was formed by the disarticulation with the hippocampus in the sagittal plane (Fig. 3.2).

The hippocampus proper volume was first manually outlined in the sagittal view and then edited and checked in the coronal view. The dorsal and anterior borders were delineated by the outer limits of the alveus and the fimbria. The anterior delineation of the hippocampus proper from the amygdaloid body was performed on the sagittal view. The infero-medial border was formed by the white matter of the subiculum. The wall of the lateral ventricle was taken as the lateral boundary, and the medial border corresponded to the adjacent cistern. The posterior limit was defined as the most caudal coronal slice where the hippocampus proper was clearly discerned in its cross-section – recognized as the junction of tail and body. Cross-sectional areas were measured from this caudal position rostrally in segments of 4 – 7 mm, up to and inclusive of the rostral limit of the head. The complete volume of the hippocampus proper was computed from the summation of measured segments (Fig. 3.3).

Figure 3.3: *Representative screen shots through the hippocampus and associated volumetry of the left hippocampus in the subject*



3.4 | Statistical analyses

A range of statistical techniques were used to better understand variation in subcortical volumetry of the hippocampus and amygdala as well as potential associations with hopelessness and learned helplessness scores within the clinical study sample. All statistical analyses were conducted in SPSS Version 25.0. Univariate descriptive statistics (means, standard deviation and variances) were computed for each age cohort (every decade of life) using a combined sex sample.

Owing to the fact that the distribution of females to males in the study samples was approximately 2:1, withthe number of males particularly limited in the clinical sample (Group 2), we chose to conduct all our analyses on the combined male and female groups. While we acknowledge that sex differences in neuroanatomical volumes and behavioural responses to stress do exist, we believe the approach used was the most salient/pragmatic given our question understudy and the limitations of the study (Ruigrok et al., 2014). Our rationale is thatthe current study is less concerned with sex differences but rather focussed on the general observation of volumetric changes associated with hopelessness and helplessness measures.

In addition, coefficients of variation (COV) and percentage differences in volume were computed for thebasis pontis, hippocampus proper and amygdaloid body. Significance testing (i.e., Analysis of variance -ANOVA) was used to test for significant differences between groups followed by post-hoc pairwise comparisons (Dunns with Bonferroni correction for multiple comparisons). Non-parametric correlations (Spearman) were used to explore any correlations between helplessness and hopelessness scores and the underlying volumetric measures of the hippocampus or amygdala. Significant testing was evaluated at $p= 0.05$.

CHAPTER 4 | RESULTS

4.1 | Volumetry of the basis pontis, amygdala and hippocampus, Group 1 (Non clinical)

4.1.1 | Basis Pontis

The results indicate that the basis pontis undergoes an increase in volume between the age cohort of 18-19 years of age (8.71 cm^3) before steadying to attain a larger mean volume of around 9.54 cm^3 between the ages of 20-29 years of age. Minimal volumetric changes in mean volume are then observed between the ages of 20 – 70 years of age (Table 4.1), with mean volume ranging between 9.33 cm^3 and 9.72 cm^3 during this time period. The percentage difference in volumes (shown in Table 4.2) indicates that the basis pontis undergoes a 9.5% increase in volumeto attain the assumed adult mean volume observed in the 20-29 age cohort.In contrast, a marked reduction in percentage volume (4.6 % reduction) is observed in the 80-89 age cohort (Table 4.2). This notable and marked decrease in volume observed in the 80 – 89 age cohort (relative to the 70 – 79 age cohort)results in a volumetric decrease basis pontis size from 9.60cm^3 to 9.16 cm^3 (Table 4.1).

4.1.2 | Amygdala

It was observed a notable age related change in mean volume of the left and right amgydala with age (Table 4.3). While mean amygdala volume ranged between 1.12cm^3 to 1.36cm^3 across ages 18-79 years of age, a marked reduction in mean volume was observed in the 80-88 age cohort, with mean volume reducing to around 1.02 cm^3. In addition, an analysis of the volume ratios of the left and right amygdala relative to basis pontis volume, revealed a similar age related change with advancing age The amygdala/basis pontis ratios varied around 0.15- 0.13 between 18-79 years of age and then dropped to 0.12 in the 80-89 age cohort (Figures 4.1 and 4.2).

Table 4.1: *Group 1 (Non clinical) age distribution by cohort (in years), mean volume of the basis pontis (cm³) and standard deviation (SD). N = sample size*

Age Cohort	**N**	**Mean volume (SD)**
18 - 19	16	8.71 (1.24)
20 - 29	70	9.54 (1.77)
30 - 39	118	9.33 (1.62)
40 - 49	113	9.62 (1.85)
50 - 59	123	9.72 (1.55)
60 - 69	121	9.67 (1.75)
70 - 79	109	9.60 (1.61)
80 - 89	30	9.16 (1.51)

Table 4.2: *Group 1(Non clinical) percentage difference in basis pontis volumes between consecutive age cohorts (in years)*

Age Cohort	**Percentage difference**
18-19 to 20-29	9.5%
20-29 to 30-39	-2.2%
30-39 to 40-49	3.2%
40-49 to 50-59	1.0%
50-59 to 60-69	-0.5%
60-69 to 70-79	-0.8%
70-79 to 80-89	-4.6%

Table 4.3: *Group 1 (Non clinical) Mean volume of the left and right amygdala (cm³). SD = standard deviation N = sample size. Age cohorts in years*

Age cohort	N	Left Amygdala Mean volume (SD)	Right Amygdala Mean volume (SD)
18 - 19	16	1.32 (0.25)	1.27 (0.27)
20 - 29	70	1.34 (0.25)	1.36 (0.27)
30 - 39	118	1.30 (0.32)	1.31 (0.30)
40 - 49	113	1.36 (0.27)	1.35 (0.32)
50 - 59	123	1.30 (0.28)	1.29 (0.29)
60 - 69	121	1.27 (0.33)	1.28 (0.30)
70 - 79	109	1.18 (0.35)	1.12 (0.31)
80 - 89	30	1.09 (0.35)	1.02 (0.32)

Figure 4.1: *Group 1 (Non clinical) bar graphs of the mean ratio of the left amygdala/basis pontis volume by age cohort (in years)*

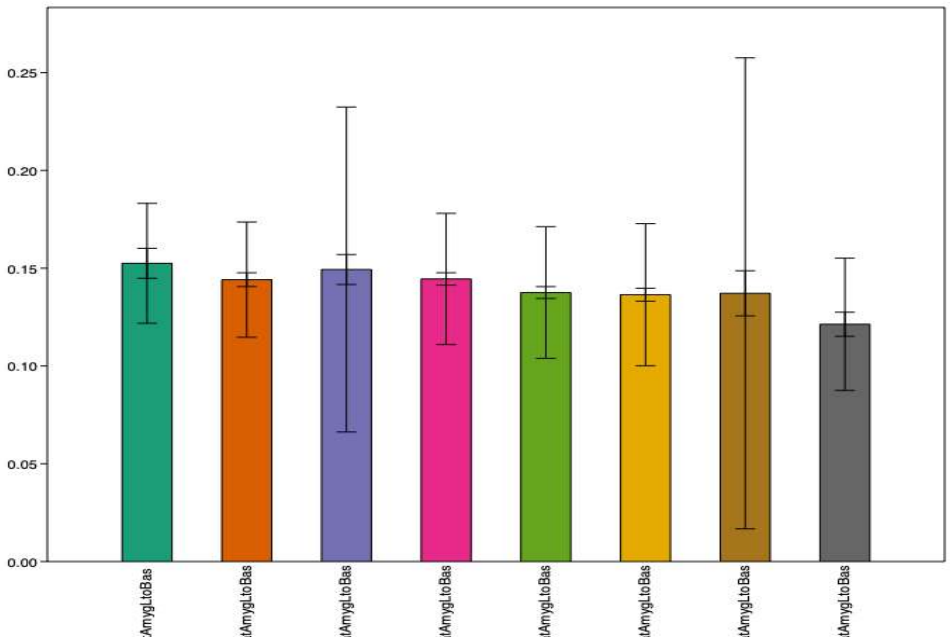
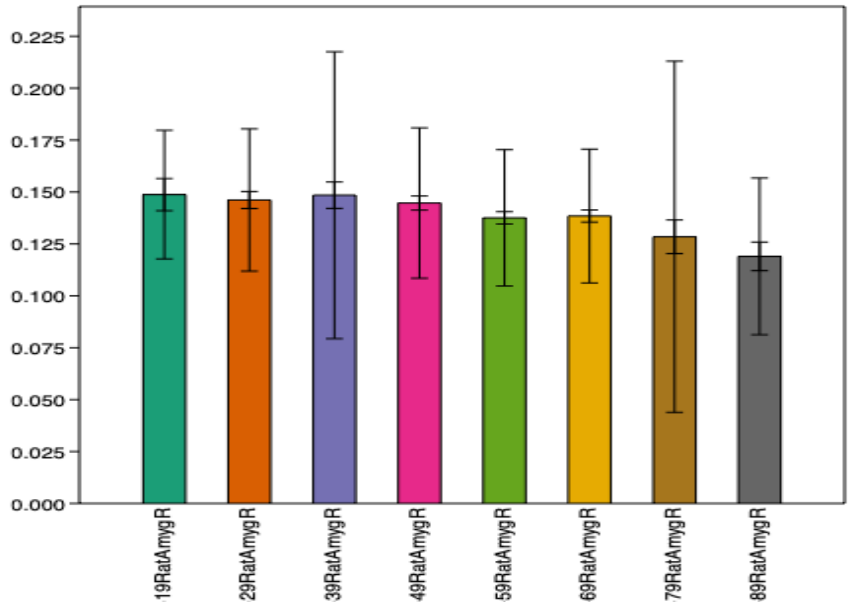


Figure 4.2: *Group 1 (Non clinical) bar graphs of the mean ratio of the right amygdala/basis pontis volume by age cohort (in years)*



4.1.3 | Hippocampus

It was noted a marked decrease in the volume of the left and right hippocampus with advancing age (Table 4.4). In the left hippocampus, volume increased from 2.28 cm^3 in the 18-19 age cohort to 2.31cm^3 in the 20-29 age cohort before undergoing a reduction to 2.19 cm^3, 2.12 cm^3 and 2.06 cm^3 in the 30-39, 40-49 and 50-59 age cohorts respectively. In the 60-69 age cohort the left hippocampal volume dropped significantly to 1.97cm^3,before reducing markedly in the 70-79 and 80-89 age cohort to values of 1.79 cm^3 and 1.59 cm^3, respectively.

Mean volume of the right hippocampus underwent a slight decrease between the 18-19 and 20-29 age cohorts (from 2.58cm^3 to 2.51cm^3), whereafter the volume steadily decreased from 2.40 cm^3 to 2.30 cm^3 in the 30-39; 40-49 and 50-59 age cohorts. By age 60-69, mean right hippocampal volume was 2.24cm^3 and underwent a further marked reduction to 2.03cm^3 in the 70-79 age cohort and dramatic reduction to 1.60cm^3in the 80-89 age cohort (Table 4.4).

Volumetric comparison of the ratio of the left and right hippocampus relative to the left and right basis pontis revealed similar results with an evident reduction in ratio commencing in the 60-69 age cohort for the left hippocampus whereas in the right hippocampus, the most evident reduction in volume ratio was observed in the 70-70 age cohort (Figures 4.3 and 4.4).

Table 4.4: *Group 1 (Non clinical) Mean volume of the left and right hippocampus (cm³). SD = standard deviation N = sample size. Age cohorts in years*

Age cohort	N	Left Hippocampus Mean Volume (SD)	Right Hippocampus Mean Volume (SD)
18 - 19	16	2.28 (0.46)	2.58 (0.51)
20 - 29	70	2.31 (0.36)	2.51 (0.42)
30 - 39	118	2.19 (0.41)	2.40 (0.40)
40 - 49	113	2.12 (0.40)	2.34 (0.45)
50 - 59	123	2.06 (0.35)	2.30 (0.48)
60 - 69	121	1.97 (0.40)	2.24 (0.46)
70 - 79	109	1.79 (0.36)	2.03 (0.42)
80 - 89	30	1.59 (0.38)	1.60 (0.38)

Figure 4.3*: Group 1 (Non clinical) bar graphs of the mean ratio of the left hippocampus/basis pontis volume by age cohort (in years)*

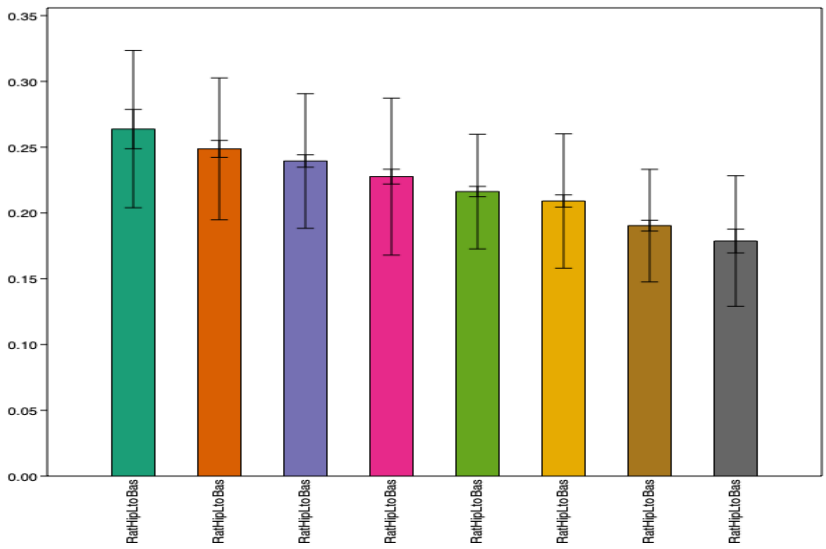
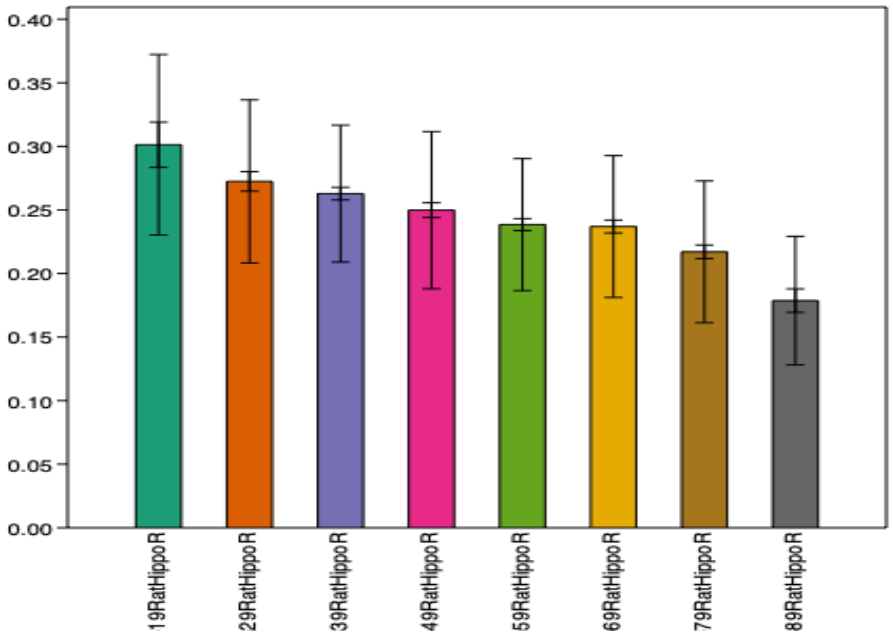


Figure 4.4: *Group 1 (Non clinical) bar graphs of the mean ratio of the right hippocampus/basis pontis volume by age cohort (in years)*



When volume ratios were compared in terms of percentage loss between consecutive age cohorts, both left and right amygdala (especially the right amygdala) and both left and right hippocampal structures (especially the right hippocampus) demonstrated significant reductions in the volume ratios from the end of the 6th decade (13% to 21%).

In the amygdala, we observed a 7.6% and 13% reduction in mean volume ratios between the 60-69 and 70-79 age cohorts respectively. This marked reduction was followed by a further reduction as volume ratios dropped by an additional 7.1% and 8.5% in the left and right amygdala respectively between the 70-79 and 80-89 age cohorts.

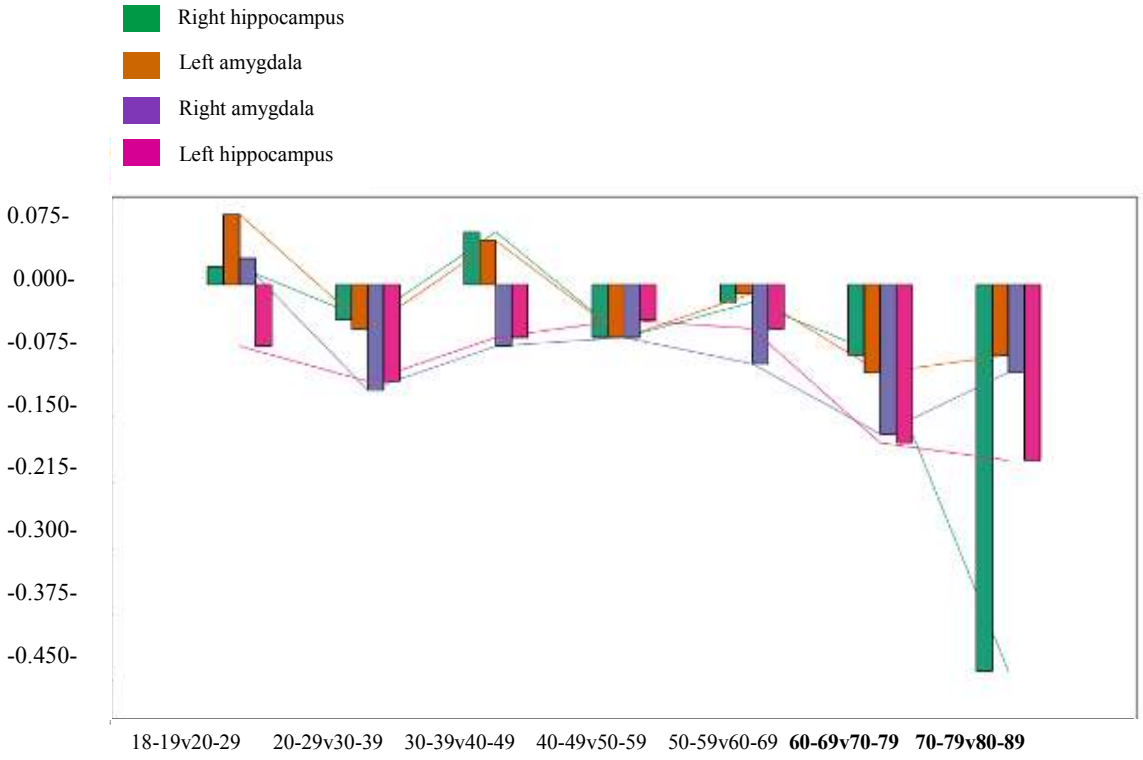
In the hippocampus, we observed a similar 9.2% and 9.6% reduction in mean volume ratios in the left and right hippocampus between the 60-69 and 70-79 age cohorts respectively. This marked reduction in hippocampal volume was followed by a further reduction as volume ratios dropped by an additional 11.3% and 21% in the left and right hippocampus respectively between the 70-79 and 80-89 age cohorts. The single most

significant involutional change was noted in the right hippocampus, where a mean volumetric reduction of 21% was observed between the 70-79 age cohort and 80-89 age cohort (Table 4.5 and Figure 4.5).

Table 4.5: *Group 1(Non clinical) percentage difference in left and right amygdala and hippocampal volumes between consecutive age cohorts. Negative values indicate a loss of mean volume relative to the previous age cohort, whereas positive values indicate a gain in mean volume relative to previous age cohort. Also shown is the percentage difference in basis pontis volume between age cohorts (in years)*

	Mean diff %	**Mean diff %**	**Mean diff %**	**Mean diff %**	**Mean diff %**
Age Cohort	**Basis Pontis**	**Left Amygdala**	**Right Amygdala**	**Left Hippocam**	**Right Hippocam**
18-19 to 20-29	9.5%	1.9%	6.6%	1.4%	-2.6%
20-29 to 30-39	-2.2%	-3.2%	-3.8%	-5.2%	-4.5%
30-39 to 40-49	3.2%	4.3%	3.6%	-3.3%	-2.6%
40-49 to 50-59	1.0%	-4.5%	-4.8%	-2.7%	-1.7%
50-59 to 60-69	-0.5%	-1.9%	-0.5%	-4.3%	-2.3%
60-69 to 70-79	-0.8%	-7.6%	-13.0%	-9.2%	-9.6%
70-79 to 80-89	-4.6%	-7.1%	-8.5%	-11.3%	-21.0%

Figure 4.5: *Bar graphs illustrating percentage volume involution of individual structures in relation to consecutive age cohorts (in years)*



4.2 | Volumetry of the amygdala and hippocampus, Group 2 (Clinical)

4.2.1 | Amygdala

In the clinical group, we observed an age related change in mean volume of the left and right amygdala (Table 4.6). Mean left amygdala volume ranged between 1.55cm³ to 1.16cm³ between ages 18- 69 years of age. Mean right amygdala volume ranged between 1.43cm³ to 1.23cm³ between ages 18- 69 years of age. In both hemipsheres, a marked reduction in mean volume was observed in the 70-79 and 80-88 age cohorts, with mean volumesin the left amygdala reducing to 1.00 cm³ and 1.11cm³ respectively. Similarly, a marked reduction in mean volume was observed in the 70-79 and 80-88 age cohortsin the right amygdala (i.e., 1.20cm³ and 1.03cm³ respectively). In addition, an analysis of the

volume ratios of the left and right amygdala relative to basis pontis volume, also revealed age related changes. The amygdala/basis pontis ratios hovered around 0.15- 0.13 between 18-79 and then dropped to 0.12 in the 80-89 age cohort (Figures 4.6 and 4.7).

Table 4.6: *Group 2 (Clinical) Mean volume of the left and right amygdala (cm³). SD = standard deviation N = sample size. Age cohorts in years*

Age cohort	N	Left Amygdala Mean volume (SD)	Right Amygdala Mean volume (SD)
18 - 19	4	1.55 (0.22)	1.43 (0.22)
20 - 29	15	1.16 (0.17)	1.25 (0.26)
30 - 39	10	1.43 (0.17)	1.42 (0.34)
40 - 49	18	1.27 (0.41)	1.23 (0.28)
50 - 59	11	1.19 (0.28)	1.43 (0.38)
60 - 69	10	1.33 (0.23)	1.24 (0.15)
70 - 79	10	1.00 (0.17)	1.20 (0.35)
80 - 89	2	1.11 (0.25)	1.03 (0.27)

Figure 4.6: *Group 2 (clinical) bar graphs of the mean ratio of the left amygdala/basis pontis volume by age cohort (in years)*

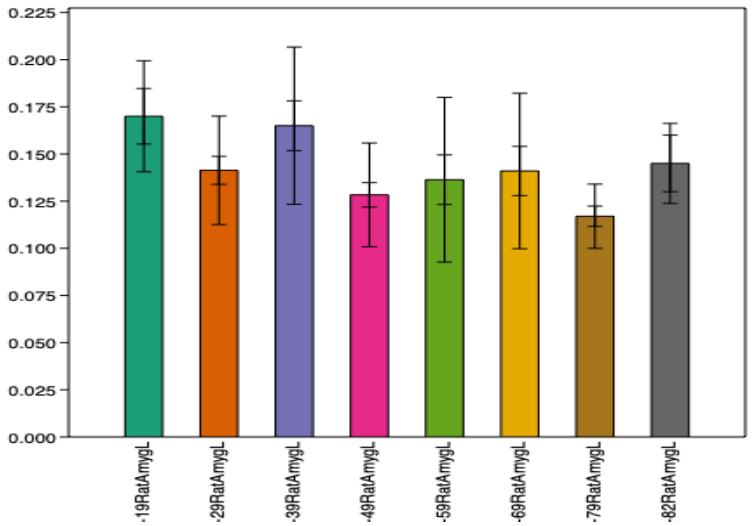
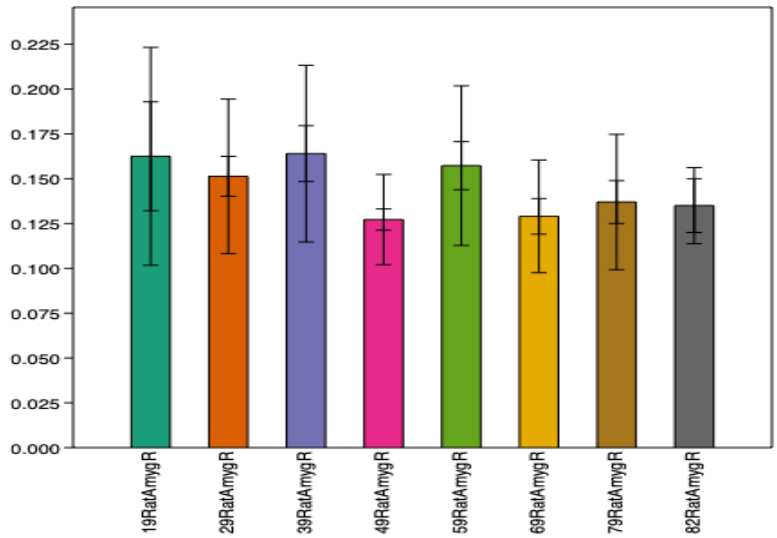


Figure 4.7: *Group 2 (clinical) bar graphs of the mean ratio of the right amygdala/basis pontis volume by age cohort (in years)*



4.2.2 | Hippocampus

In the clinical sample (Group 2), we noted a marked decrease in the volume of the left and right hippocampus with advancing age (Table 4.7). In the left hippocampus, volume ranged between 2.27cm³ and 1.93cm³ between the 18-19 and 60-69 age cohorts; however, a marked reduction in left hippocampal volume was observed in the 70-79 and 80-89 age cohorts as hippocampal volumes reduced to 1.64cm³ and 1.29cm³, respectively. Similarly, for the right hippocampus, mean volume ranged between 2.16cm³ and 2,54cm³ between 18-19 and 60-69 age cohort; however, in the 70-79 and 80-89 age cohorts we observed a marked reduction in right hippocampal volume to 2.07cm³and 1.67cm³, respectively (Table 4.7). Volumetric comparison of the ratio of the left and right hippocampus relative to the left and right basis pontis revealed similar results with an evident reduction in ratio commencing in the 60-69 age cohort for the left hippocampus whereas in the right hippocampus, the most evident reduction in volume ratio was observed in the 70-70 age cohort (see Figures 4.8 and 4.9).

Table 4.7: *Group 2 (Clinical) Mean volume of the left and right hippocampus (cm³). SD = standard deviation N = sample size. Age cohort in years.*

Age cohort	N	Left Hippocampus Mean volume (SD)	Right Hippocampus Mean volume (SD)
18 - 19	4	2.27 (0.32)	2.46 (0.59)
20 - 29	15	2.03 (0.24)	2.54 (0.31)
30 - 39	10	2.17 (0.46)	2.54(0.73)
40 - 49	18	1.92 (0.37)	2.21 (0.34)
50 - 59	11	1.93 (0.37)	2.16 (0.46)
60 - 69	10	2.12 (0.47)	2.29 (0.40)
70 - 79	10	1.64 (0.30)	2.07 (0.42)
80 - 89	2	1.29 (0.49)	1.67 (0.79)

Figure 4.8: *Group 2 (clinical) bar graphs of the mean ratio of the left hippocampus/basis pontis volume by age cohort (in years)*

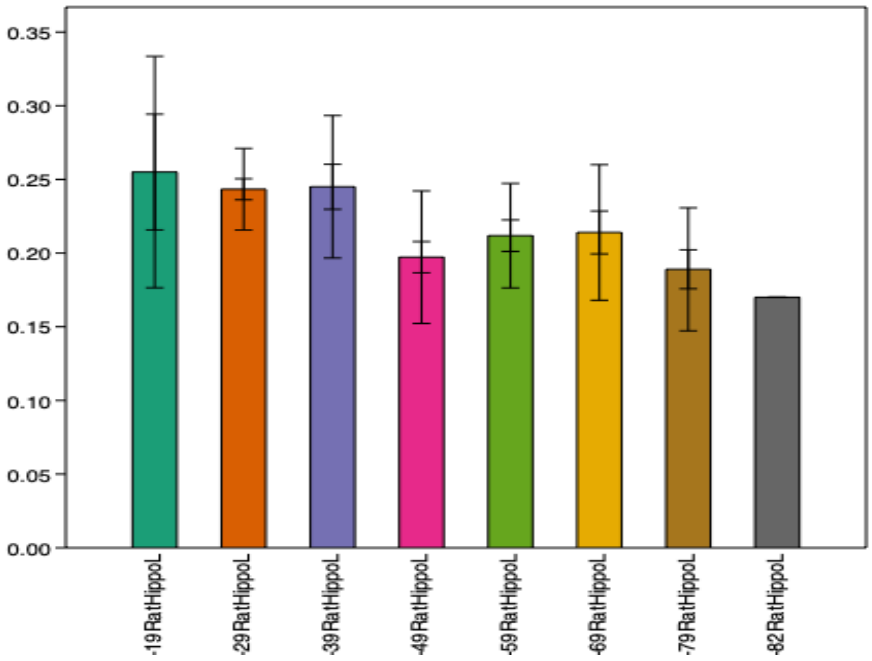
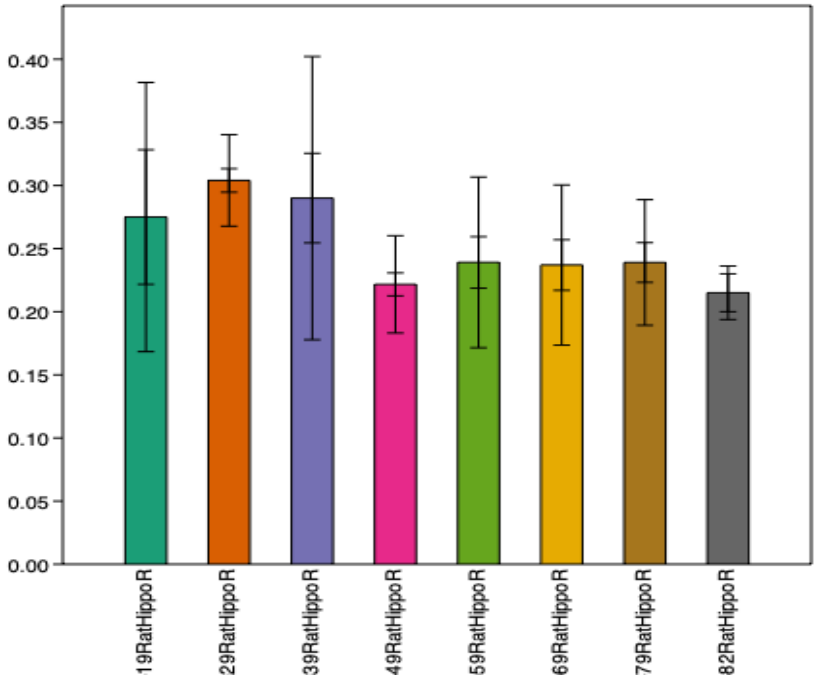


Figure 4.9: *Group 2 (clinical) bar graphs of the mean ratio of the right hippocampus/basis pontis volume by age cohort (in years)*



4.3 | BHI and LHS scores

BHI scores reflecting degrees of hopelessness were noted to be higher in the younger (18-29) and in the oldest age cohorts(Table 4.8). Similarly LHS scores reflecting degrees of learned helplessness were higher in the younger age cohorts (18-29) (Table 4.8). Correlation analysis (Spearman) revealed that hopelessness and learned helplessness scores were significantlycorrelated with one another (p=1.89x10^{-08}) across all age cohorts(Table 4.9). An analysis of the mean volume ratios across all age cohorts revealed most significant correlations between diminished volume ratios of the left amygdala and the LHS (p=0.02498) and between diminished volume ratios of the left hippocampus and the LHS (p=0.030928)(Table 4.9). Lesser degrees of significance were noted in the correlations of volume ratios of the right hippocampus with the LHS (p=0.044647) and the left hippocampus with the BHI (p=0.045459). The significance value of the diminished volume ratios of the left hippocampus and the LHS is also depicted in Figure 4.10.

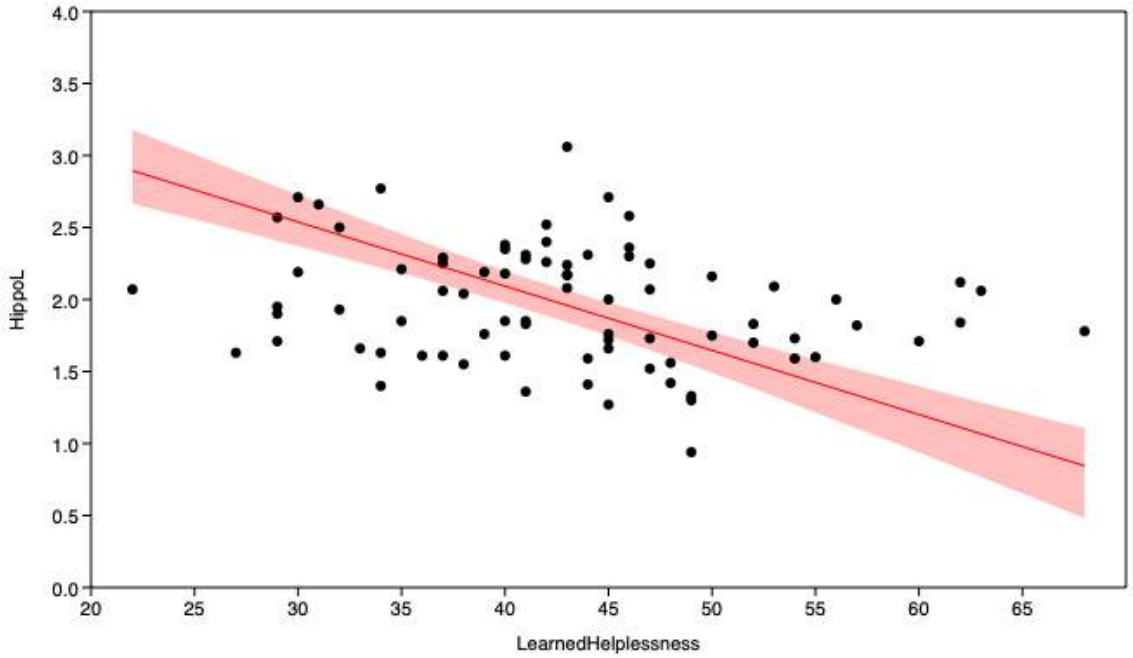
Table 4.8: *Group 2 (Clinical) Mean Score of the BHI and LHS by age cohort. SD = standard deviation N = sample size. Age cohort in years*

Age Cohort	N	Mean Score BHI (SD)	Mean Score LHS (SD)
18 - 19	4	6.5 (7.05)	48.75 (14.31)
20 - 29	15	6.33 (5.96)	46.20 (9.33)
30 - 39	10	4.50 (5.72)	40.70 (9.94)
40 - 49	18	3.78 (4.75)	40.83 (9.29)
50 - 59	11	4.73 (4.58)	43.09 (8.95)
60 - 69	10	3.30 (2.31)	41.30 (5.10)
70 - 79	10	3.90 (4.84)	42.80 (8.47)
80 - 89	2	9.50 (7.78)	41.50 (10.61)

Table 4.9: *Group 2 (Clinical) Significant P values of mean volume ratios versus BHI and LHS scores across all age cohorts*

Variable	p Values (Spearman D)
Left hippocampus vs BHI	0.045459
Left amygdala vs LHS	0.024980
Left hippocampus vs LHS	0.030928
Right hippocampus vs LHS	0.044647
BHI vs LHS	$1.89x10^{-08}$

***Figure 4.10**: The correlation of decreasing left hippocampal mean volumes with mean increased LHS scores (Spearman D)*



An analysis of the data of individual age cohorts revealed correlations of significance in both volume ratios and degrees of hopelessness and helplessness as well as in the co-existence of hopelessness and helplessness (Table 4.10). Correlation analyses (Spearman rho) revealed a significant negative correlation of mean left hippocampus proper volume and mean BHI scores in the 40-49 age cohort (r= -0.60728; p = 0.0075203) such that 37% of the variation in BHI could be explained by left hippocampal volume (Table 4.10). A correlation of significance was also noted for the mean right amygdala volume and the mean BHI in the 70-79 age cohorts (r= 0.83166; p = 0.0028525) such that 69% of the variation in BHI could be explained by right hippocampal volume (Table 4.10)

Further correlations were noted with the left hippocampus and helplessness score in the 40-49 cohort (p=0.040369), right hippocampus and hopelessness score in the 40-49 cohort (p=0.042822), right amygdala and helplessness score in the 60-69 cohort (p=0.030211) and left amygdala and hopelessness score in the 30-39 cohort

(p=0.030798).Significant co-existences of hopelessness and helplessness scores were noted in the 20-29 cohort (p=0.00063734), 40-49 cohort (P=0.013408) and the 50-59 cohort (p=0.01328).There were no correlations of significance found between the differences in volume ratios of the right and left hippocampus and the scores of BHI and LHS.

Table 4.10: *Group 2 (Clinical) Significant P, R and R2 values of mean volume ratios versus BHI and LHS scores across age cohorts (in years)*

Age Cohort	Variable	R (Spearmans rho)	R2 (Spearmans rho)	p Values (Spearmans rho)
20-29	BHI vs LHS	0.77798	0.60525288	0.00063734
30-39	Left amygdala vs BHI	-0.67913	0.461217557	0.030798
40-49	Left hippocampus vs BHI	-0.60728	0.368788998	0.0075203
40-49	Right hippocampus vs BHI	-0.48197	0.232295081	0.042822
40-49	Left hippocampus vs LHS	-0.48705	0.237217703	0.040369
40-49	BHI vs LHS	0.57057	0.325550125	0.013408
50-59	BHI vs LHS	0.71560	0.51208336	0.01328
60-69	Right amygdala vs LHS	0.51064	0.26075321	0.030211
70-79	Right amygdala vs BHI	0.83166	0.691658356	0.0028525

CHAPTER 5 | DISCUSSION

The current study aimed to investigate whether variations in the size of the amygdala and hippocampus proper across ages correlated with the feelings of hopelessness and learned helplessness and whether this predisposed to the development of neurodegenerative diseases such as Alzheimer's disease. In order to test these possibilities, the size of the amygdala and hippocampus proper, normalized to the size of the basis pontis, was first investigated in a large cohort of non-clinical subjects across ages. This was followed by the study of these structures in clinical patients, with the derived ratios being compared to scores of hopelessness and learned helplessness across ages compared to age-matched groups.

The primary results of the non-clinical group (Group 1) investigated in this this study include: 1) the volume of the basis pontis remains relatively constant until the eighth decade; 2) the volume ratios of the amygdala and hippocampus remain relatively constant until the end of the sixth decade, following which there are accelerated involutional changes; and 3) the volume ratio of the right hippocampus proper remains consistently greater than the left. In the clinical group (Group 2) investigated it was observed that: 1) the volume ratios of the left hippocampus and left amygdala diminish with increased learned helplessness scores; 2) the left hippocampus volume diminishes with increased hopelessness; 3) the right hippocampus volume diminishes with increased helplessness; and 4) the emotional states of hopelessness and learned helplessness co-exist significantly.

5.1 | Volumetry of basis pontis, amygdala and hippocampusin Group 1 (Non clinical)

5.1.1 | Basis pontis

As the basis pontis was selected as the reference volumefor comparison to structure of interest, initial studies evaluated the volume of the basis pontis through the age group ranges

in Group 1. The finding that the volume of the basis pontis remains relatively constant until the eighth decade validates the selection of the basis pontis as a reference volume for use in the current study, in agreement with similar findings of the minimal involution of the anterior pons with increasing age (Raz et al., 2001). Biologically and clinically it becomes apparent that prevailing influences predisposing to atrophy of the cortex as well as to atrophy of many subcortical structures apply only minimally to the basis pontis. It is proposed that creating ratios of volumes of telencephalic structures relative to the basis pontis for correlative volumetry, is more accurate than generating whole brain-based corrected volumes (Kijonka, et al., 2020).

5.1.2 | Amygdala

The volume ratios of the left and right amygdala revealed the expected age related involution with advancing age (Fujita et al., 2023) (Table 4.3, Figs. 4.1, 4.2). The data derived from Group 1 revealed a significant accelerated involution of the volume of the amygdala from the end of the 6th decade, in agreement with previous studies (Fujita et al., 2023). For reasons unknown, the progression was noted to be minimal in the left amygdala (Table 4.3, Fig.4.1). The comparative volume ratios of the left and right amygdala showed parity across all age cohorts until the end of the sixth decade. Progressive increased atrophy of the right amygdala was noted relative to the left in the seventh and eighth decades. Mild and subjective cognitive decline as opposed to normal cognitive function have been found to be associated with decreased volume of the right amygdala relative to the left (Yue, et al., 2018). The findings in this study may reflect an increased incidence of mild and subjective cognitive impairment in the seventh and eighth decades.

5.1.3 Hippocampus proper

The comparative volume ratios of the left and right hippocampus proper indicated increased volume ratios of the right hippocampus proper relative to the left across the age cohorts, consistent with previous studies (Honeycutt & Smith, 1995). A significant decreasing volumetric ratio of the left hippocampus proper was noted as commencing in the 60-69 age cohort. Increased atrophy of the left hippocampus relative to the right has been noted in association with mild and subjective cognitive dysfunction relative to normal function (Yue, et al., 2018). In the current study the decreasing volume of the right hippocampus proper commenced in the 70-79 age cohort. Significant right hippocampal atrophy has been shown to occur in association with Parkinson's disease (Uribe, et al., 2018).

In summary, when volume ratios were compared in terms of percentage loss between consecutive age cohorts, the right amygdala and both hippocampal structures demonstrated significant involution from the end of the 6th decade (13% to 21%). The most significant involutional change was noted in the right hippocampus (Table 4.4, Figure 4.4). A negative figure indicates loss of mean volume relevant to the previous age cohort.

Based on existing studies, the configurations of the amygdala and hippocampus proper observed in this study potentially reflect increasing degrees of cognitive dysfunction in the seventh decade followed by a significant increase in the occurrence of Parkinson's disease towards the end of the seventh decade (Yue, et al., 2018; Uribe, et al., 2018). A possible correlation with the observed configuration of amygdala and hippocampus proper atrophy is an increased level of chronic depression occurring in the psycho-social environment of these age cohorts (Oyarce et al., 2020; Tottenham &Sheridan, 2010).

5.2 | Volumetry of the amygdala and hippocampus, Group 2 (Clinical)

5.2.1 | Amygdala

The mean volumes of the left and right amygdala were comparable across all age cohorts. The volume ratios of the left and right amygdala revealed diminished volume ratios in the 60-69 and 70-79 age cohorts. Overall involutional trends were not as clearly defined as in sample 1, possibly due to the smaller sample size comprising Group2 (Table 4.6, Figs. 4.6, 4.7).

5.2.2 | Hippocampus proper

The comparative volume ratios of the left and right hippocampus proper indicated increased volume ratios of the right hippocampus proper relative to the left across the age cohorts, consistent with previous studies (Honeycutt & Smith, 1995). A decreased volumetric ratio of the left and right hippocampus proper was noted in the 40-89 year cohorts relative to the volume ratios of the 18-39 year cohorts (Table 4.7, Figs. 4.8, 4.9). Again, the overall involutional trends were not as clearly defined as in Group 1, possibly due to the smaller sample size comprising Group 2.

5.3 | BHI and LHS Scores

BHI scores reflecting degrees of hopelessness were noted to be higher in the younger age cohorts (18-29) and in the oldest age cohort (80-89). The oldest cohort had less significance due to the low number of subjects investigated (Table 4.8). Similarly LHS scores reflecting degrees of learned helplessness were higher in the younger age cohorts (18-29) (Table 4.8). An analysis of the score results of the BHI and LHS across all age cohorts revealed a highly significant correlation (p=1.89x10^{-08}) indicating the probability of the co-existence of hopelessness and learned helplessness (Table 4.9).

5.4 | Senescence and brain atrophy

The atrophy of the brain during senescence is regarded as a normal aging process; however several pathological processes have been identified which have been shown to influence the progression of senile volume loss.Evidence indicates that cerebral atrophy may be caused by the sensitization of microglia and chronic cerebral inflammation (Niraula et al., 2017), with a significant correlation between sensitized microglia and chronic inflammation in adulthood associated with childhood adversity (Chen et al., 2021). Social isolation has also been recognised as contributing to the increased cerebral atrophy of senescence (Lammer et al., 2023). Of relevance to the current study, the state of learned helplessness has been shown to be correlated with theatrophy of the hippocampus proper possibly due to the decrease of adult hippocampal neurogenesis in an environment experiencing chronic inflammation (Bourgognon & Cavanagh, 2020; Malberg & Duman, 2003).

The finding in this study of the significant increased atrophy of the right and left hippocampus proper in relation to the right and left amygdala in Group 1 may reflect this element. In a bio-psycho-social context, degrees of atrophy of the amygdaloid body and hippocampus proper in the advanced age cohorts may reflect a pathological process of chronic inflammation with multiple potential aetiologies influencing the activation and progression of the inflammation.

5.5 | Volumetry in regard to hopelessness and learned helplessness

Based on psychological studies the interpretation of hopelessness and learned helplessness is that together they may precipitate clinical depression, sustain it, and determine the effectiveness of intervention. (Henkel et al., 2002). Hopelessness may be further defined as a cognitive vulnerability that evolves into depression when exposed to a stressful life event (Haeffel et al., 2017). An important consideration in regard to these emotional states is that

they have been shown to influence the volumes of the amygdaloid body and hippocampus proper (McEwen, 1998; Malberg & Duman, 2003; Oyarce et al., 2020).

Learned helplessness has been shown to give rise to atrophy of the hippocampus proper possibly on the basis of decreased adult neurogenesis (Malberg & Duman, 2003). Depression and anxiety, when occurring together, predispose individuals to atrophy of the hippocampus proper (Oyarce et al., 2020; McEwen, 1998). In a percentage of individuals the coexistence of anxiety limits the degree of hippocampus proper atrophy, possibly indicating a period prior to amygdaloid body atrophy (Oyarce et al, 2020).Studies have revealed that excessive hippocampal proper atrophy relative to normal mean volumes for age is the most consistent predictor of the progression to Alzheimer's disease (Henneman, 2009). The integration of these observations leads to the implication that hopelessness and learned helplessness predispose individuals to the development of Alzheimer's disease at least on the basis of hippocampus proper atrophy.

Studies of children reared in adverse conditions associated with nurture deprivation reveal significant hypertrophy of the amygdaloid body when compared with control groups (Gilhooly et al., 2010). While increased amygdaloid body volumes have been correlated with depression, a significant percentage of individuals living with chronic depression show progressive amygdaloid body atrophy (Tottenham &Sheridan, 2010). Atrophy of the amygdaloid body is noted to be a consistent trait of Alzheimer's disease (Nikolenko et al., 2020).

It thus becomes apparent that the states of hopelessness and learned helplessness combined, underpin and sustain chronic depression. Chronic depression with or without anxiety predisposes individuals to an increased risk of developing Alzheimer's disease. This increased risk associated with depression may as a consequence directly contribute to the atrophy of the amygdaloid body and hippocampus proper.

In this study, the most significant relevant finding was that of the correlation of the mean scores of the BHI and the LHS (p=1.89x10^{-08}) across the mean of all age cohorts of Group 2. The statistical significance in this context refers to the scores of the BHI and LHS that fluctuate in tandem with each other.

The mean values, however, of the individual scores of the BHI and the LHS in each cohort were not significant in that the BHS scores were within the mild range and the LHS scores were within a range well below one standard deviation above the mean of 39.12 as indicated previously (Beck et al., 1974; Quinless & Nelson, 1988). Notwithstanding the low scores of the BHI and the LHS, the significant correlation contributes to the proposed concept that these two parameters together may underpin depression at higher scores.

While hopelessness and learned helplessness have been shown to be contributing core elements to depression, they appear to have different individual influences upon volumetric ratios. The most significant influence upon volumetric ratios across all age cohorts of Group 2 were reflected in the correlation of raised LHS scores with diminished volume ratios of the left hippocampus proper (p=0.030928) and the left amygdaloid body (p=0.02498). However the overall significance of these findings diminishes in the face of the relatively low mean score values but at the least indicate a possible influence of hopelessness and learned helplessness upon the progression of atrophy in the amygdaloid body and hippocampus proper.

5.6 | Shortcomings and limitations

The purpose of this study was to initially perform a volumetric analysis of the amygdaloid body and hippocampus proper. This reference quantification was then applied to a clinical cohort in which the influences of the states of hopelessness and learned helplessness upon the amygdaloid body and hippocampus proper were evaluated. A significant correlation

of increased scores of hopelessness and learned helplessness with a decreased volume of the hippocampus proper and to a lesser degree, the amygdaloid body, could be considered to predispose individuals to heightened risk in the development of Alzheimer's disease.

The scores of the BHI and LHS found in the Group 2 cohort are in the mild range for the BHI and less than one standard deviation of the mean in the LHS. These low scores decrease the overall significance of the influences of hopelessness and learned helplessness upon atrophic changes in the amygdaloid body and hippocampus proper.

Further studies are required in which baseline and clinical cohorts comprising individuals with chronic depression, are evaluated. This is based on the assumption that the scores of the BHI and LHS would be significantly higher. A longitudinal study would further serve to define the findings of the current study.

5.7 | Conclusions

The current study has confirmed the constancy of the volume of the basis pontis from the second decade to the end of the seventh decade. The significance of the volume of the basis pontis as a reference for volume ratios has been validated. The relative constancy of the volumes of the amygdaloid body and hippocampus proper up to the end of the sixth decade followed by a significant decrease in the seventh decade has also been confirmed. Indications have been noted that degrees of hopelessness and learned helplessness may predispose individuals to amygdaloid body and hippocampus proper atrophy, but the significance of this finding is decreased in the face of decreased scores derived from the BHI and LHS. Further studies are required to validate this hypothesis.

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Appendices

Appendix 1: Ethical Clearance Certificates

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Dr I Weinberg
School of Anatomical Sciences
Medical School
University

E-mail: quantum@gam.co.za

CC: Supervisor: Professor P Manger
<Paul Manager@wits.ac.za>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2023/04/28

REF: R14/49

PROTOCOL NO: **M230110** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Amygdaloid and hippocampal volumetric variations and the effects of the feelings of hopelessness and helplessness*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG

R49 Dr I Weinberg

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M230110

NAME: (Principal Investigator)	Dr I Weinberg
DEPARTMENT:	School of Anatomical Sciences Medical School University
PROJECT TITLE:	*Amygdaloid and hippocampal volumetric variations and the effects of the feelings of hopelessness and helplessness*
DATE CONSIDERED:	2023/01/27
DECISION:	Approved unconditionally
CONDITIONS:	This approval covers Part 1 of the study only (record review); Part 2 is to be discussed with the HREC (Medical) at, or close to, the completion of Part 1
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professor P Manger
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/04/28

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **January** and therefore reports and re-certification will be due in the month of **January** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

2 MAY 2023
Date

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr I Weinberg

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M230110

NAME: (Principal Investigator)	Dr I Weinberg
DEPARTMENT:	School of Anatomical Sciences Medical School University
PROJECT TITLE:	*Amygdaloid and hippocampal volumetric variations and the effects of the feelings of hopelessness and helplessness*
DATE CONSIDERED:	2023/01/27
DECISION:	Approved unconditionally
CONDITIONS:	Previous condition (approval of Part 2 of the study) satisfied on 2023/06/13
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professor P Manger
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/04/28 **EXPIRY DATE:**

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **January** and therefore reports and re-certification will be due in the month of **January** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

Date 13/6/23

Appendix 2:BHI Questionnaire

Part 1: Please insert a X in the appropriate box where the first box is 'true' and the second box is 'false'.

	True	False
1. I look forward to the future with hope and enthusiasm.	☐	☐
2. I might as well give up because I can't make things better for myself.	☐	☐
3. When things are going badly, I am helped by knowing they can't stay that way forever.	☐	☐
4. I can't imagine what my life would be like in 10 years.	☐	☐
5. I have enough time to accomplish the things I most want to do.	☐	☐
6. In the future, I expect to succeed in what concerns me most.	☐	☐
7. My future seems dark to me.	☐	☐
8. I expect to get more of the good things in life than the average person.	☐	☐
9. I just don't get the breaks, and there's no reason to believe I will in the future.	☐	☐
10. My past experiences have prepared me well for my future.	☐	☐
11. All I can see ahead of me is unpleasantness rather than pleasantness.	☐	☐

12. I don't expect to get what I really want. ☐ ☐

13. When I look ahead to the future, I expect I will be happier than I am now. ☐ ☐

14. Things just won't work out the way I want them to. ☐ ☐

15. I have great faith in the future. ☐ ☐

16. I never get what I want so it's foolish to want anything. ☐ ☐

17. It is very unlikely that I will get any real satisfaction in the future. ☐ ☐

18. The future seems vague and uncertain to me. ☐ ☐

19. I can look forward to more good times than bad times. ☐ ☐

20. There's no use in really trying to get something I want because I probably won't get it ☐ ☐

Scoring

0-3 None or minimal

4-8 Mild

9-14 Moderate. May not be in immediate danger, but requires frequent regular monitoring.

15+ Severe.

Appendix 3: LHS Questionnaire

Strongly agree Agree Disagree Strongly disagree



1. No matter how much energy I put into a task, I feel I have no control over the outcome.
2. I feel that my ability to solve problems is the cause of my success.
3. I can find solutions to difficult problems.
4. I don't place myself in situations in which I cannot predictthe outcome.
5. If I complete a task successfully, it is probably because of my ability.
6. I have the ability to solve most of life's problems.
7. When I do not succeed at a task, I do not attempt any similar tasksbecause I feel that I would fail them also.
8. When something doesn't turn out the way I planned,I know it is because I didn't have the ability to start with.
9. Other people have more control over their success and/or failure than I do.
10. I try new tasks if I have failed similar ones in the past.
11. When I perform poorly, it is because I don't have the ability to perform better.
12. I accept tasks even if I am not sure that I will succeed at them.

13. I feel that I have little control over the outcomes of my work. ☐ ☐ ☐ ☐

14. I am successful at most tasks I try. ☐ ☐ ☐ ☐

15. I feel that anyone else could be better than me at most tasks. ☐ ☐ ☐ ☐

16. I am able to reach my goals in life. ☐ ☐ ☐ ☐

17. When I don't succeed at a task, I find myself blaming my own stupidity for my failure. ☐ ☐ ☐ ☐

18. No matter how hard I try, things never seem to work out the way I want them to. ☐ ☐ ☐ ☐

19. I feel that my success reflects my ability, not chance. ☐ ☐ ☐ ☐

20. My behaviour seems to influence the success of a work group. ☐ ☐ ☐ ☐

Learned Helplessness Questionnaire – Score Key

The scoring key for the LHS Form B is described below. Items # 1, 4, 7, 8, 9. 11, 13, 15, 17, and 18 are scored as follows: Strongly Agree= 4 Agree= 3 Disagree= 2 Strongly Disagree= 1 Items # 2, 3, 5, 6, 10, 12, 14, 16, 19, and 20 are scored as follows: Strongly Agree = 1 Agree = 2 Disagree = 3 Strongly Disagree = 4 Add all twenty item scores to obtain the final score.

The possible range of scores on the LHS is 20 to 80 with higher scores indicating higher learned helplessness.

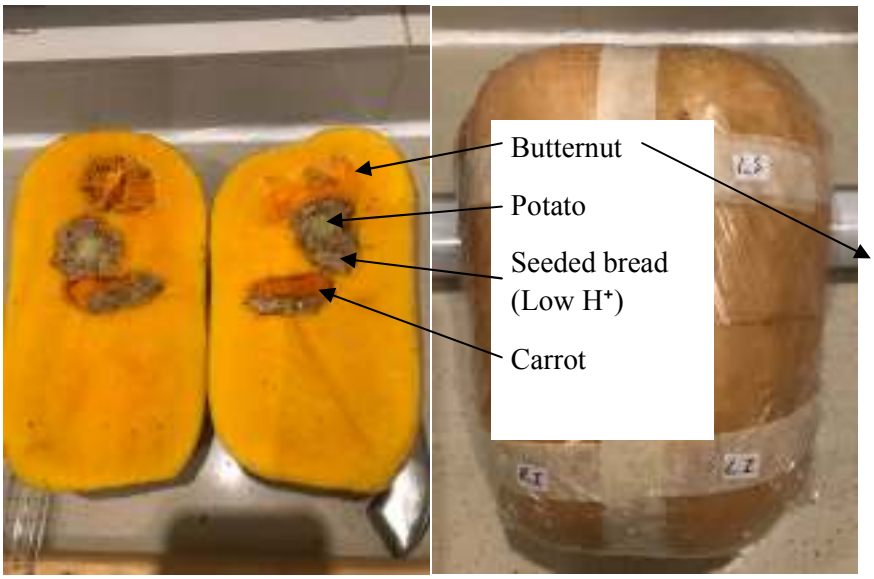
Appendix 4: Volume displacement correlation

Description

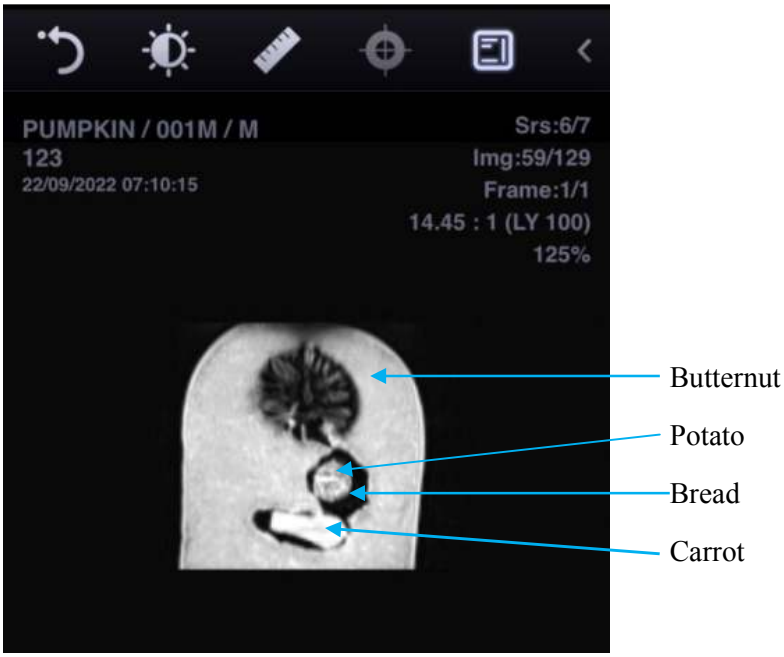
A model was constructed from vegetable matter to depict the amygdala and hippocampus on the left and right sides. The actual water displacement volume of the entities was initially established. Thereafter the entire construct was MRI scanned and the volumes calculated using Lambert's formula incorporated in the MRI volumetric tool. The MRI volumetric calculations approximated with a significant degree of accuracy the actual volume displacements.

Actual volume displacements			**MRI calculations**		
Amygdala	L= 2.0	R= 2.5	Amygdala	L= 1.96	R= 2.44
Hippocampus	L= 2.5	R= 3.0	Hippocampus	L= 2.5	R= 3.16

Scanning model



MRI of model


PUMPKIN / 001M / M
123
22/09/2022 07:10:15
Srs:6/7
Img:59/129
Frame:1/1
14.45 : 1 (LY 100)
125%
Butternut
Potato
Bread
Carrot

Appendix 5: Data

Table A5-1: Group 1 – non clinical data. Age cohorts in years.

Age Cohort	Variable	N	Min	Max	Mean	Standard deviation
18-19	Age	16	18.00	19.00	18.69	0.48
	BasPon	16	7.07	11.22	8.71	1.24
	AmygL	16	0.84	1.61	1.32	0.25
	RatioAmygLtoBasPon	16	0.10	0.21	0.15	0.03
	HippoL	16	1.57	3.51	2.28	0.46
	RatioHippoLtoBasPon	16	0.17	0.39	0.26	0.06
	AmygR	16	0.65	1.72	1.27	0.27
	RatioAmygRtoBasPon	16	0.10	0.20	0.15	0.03
	HippoR	16	1.89	3.55	2.58	0.51
	RatioHippRToBasPon	16	0.19	0.40	0.30	0.07
20-29	Age	70	20.00	29.00	24.81	2.91
	BasPon	70	6.16	13.93	9.54	1.77
	AmygL	70	0.81	2.01	1.34	0.25
	RatioAmygLtoBasPon	70	0.10	0.21	0.14	0.03
	HippoL	70	1.47	3.39	2.31	0.36
	RatioHippoLToBasPon	70	0.12	0.40	0.25	0.05
	AmygR	70	0.80	2.04	1.36	0.27
	RatioAmygRToBasPon	70	0.10	0.24	0.15	0.03
	HippoR	70	1.60	3.48	2.51	0.42
	RatioHippRToBasPon	70	0.13	0.42	0.27	0.06
30-39	Age	118	30.00	39.00	35.23	2.71
	BasPon	118	6.85	15.72	9.33	1.62
	AmygL	118	0.68	2.70	1.30	0.32
	RatioAmygLtoBasPon	118	0.08	0.96	0.15	0.08
	HippoL	118	1.27	3.44	2.19	0.41
	RatioHippoLToBasPon	118	0.11	0.38	0.24	0.05
	AmygR	118	0.63	2.78	1.31	0.30
	RatioAmygRToBasPon	118	0.10	0.80	0.15	0.07
	HippoR	118	1.45	3.55	2.40	0.40
	RatioHippRToBasPon	118	0.14	0.45	0.26	0.05
40-49	Age	113	40.00	49.00	44.53	2.96
	BasPon	113	6.05	16.38	9.62	1.85
	AmygL	113	0.79	2.39	1.36	0.27
	RatioAmygLtoBasPon	113	0.09	0.25	0.14	0.03
	HippoL	113	1.24	3.15	2.12	0.40
	RatioHippoLToBasPon	113	0.11	0.42	0.23	0.06
	AmygR	113	0.85	2.79	1.35	0.32
	RatioAmygRToBasPon	113	0.10	0.26	0.14	0.04
	HippoR	113	1.17	3.46	2.34	0.45
	RatioHippRToBasPon	113	0.12	0.44	0.25	0.06

50-59	Age	123	50.00	59.00	54.74	2.85
	BasPon	123	6.46	13.73	9.72	1.55
	AmygL	123	0.48	2.15	1.30	0.28
	RatioAmygLtoBasPon	123	0.05	0.29	0.14	0.03
	HippoL	123	1.27	3.38	2.06	0.35
	RatioHippoLToBasPon	123	0.11	0.35	0.22	0.04
	AmygR	123	0.51	2.15	1.29	0.29
	RatioAmygRToBasPon	123	0.05	0.26	0.14	0.03
	HippoR	123	0.62	4.83	2.30	0.48
	RatioHippRToBasPon	123	0.07	0.42	0.24	0.05
60-69	Age	121	60.00	69.00	64.30	2.74
	BasPon	121	5.95	15.42	9.67	1.75
	AmygL	121	0.70	2.17	1.27	0.33
	RatioAmygLtoBasPon	121	0.07	0.24	0.14	0.04
	HippoL	121	1.10	3.59	1.97	0.40
	RatioHippoLToBasPon	121	0.10	0.36	0.21	0.05
	AmygR	121	0.63	2.16	1.28	0.30
	RatioAmygRToBasPon	121	0.09	0.23	0.14	0.03
	HippoR	121	1.02	3.44	2.24	0.46
	RatioHippRToBasPon	121	0.11	0.37	0.24	0.06
70-79	Age	109	70.00	79.00	73.81	2.96
	BasPon	109	5.10	13.73	9.60	1.61
	AmygL	109	0.52	2.72	1.18	0.35
	RatioAmygLtoBasPon	109	0.03	1.34	0.14	0.12
	HippoL	109	0.75	3.00	1.79	0.36
	RatioHippoLToBasPon	109	0.10	0.29	0.19	0.04
	AmygR	109	0.40	2.12	1.12	0.31
	RatioAmygRToBasPon	109	0.03	0.94	0.13	0.08
	HippoR	109	0.72	2.75	2.03	0.42
	RatioHippRToBasPon	109	0.10	0.40	0.22	0.06
80-88	Age	30	80.00	88.00	83.63	2.19
	BasPon	30	6.73	12.41	9.16	1.51
	AmygL	30	0.36	1.93	1.09	0.35
	RatioAmygLtoBasPon	30	0.05	0.18	0.12	0.03
	HippoL	30	0.72	2.30	1.59	0.38
	RatioHippoLToBasPon	30	0.10	0.31	0.18	0.05
	AmygR	30	0.51	1.64	1.02	0.32
	RatioAmygRToBasPon	30	0.06	0.21	0.12	0.04
	HippoR	30	0.88	2.50	1.60	0.38
	RatioHippRToBasPon	30	0.10	0.29	0.18	0.05

Table A5-2: Group 2 - clinical data where Part 1 is the BHI score and Part 2 the LHS score. Age cohorts in years.

Age Cohort	Variable	N	Min	Max	Mean	Standard deviation
18-19	Age	4	18.00	19.00	18.25	0.50
	BasPon	4	6.45	10.28	9.26	1.87
	AmygL	4	1.33	1.79	1.55	0.22
	RatioAmygLtoBasPon	4	0.14	0.21	0.17	0.03
	HippoL	4	2.00	2.71	2.27	0.32
	RatioHippoLToBasPon	4	0.19	0.36	0.26	0.08
	AmygR	4	1.12	1.59	1.43	0.22
	RatioAmygRToBasPon	4	0.11	0.25	0.16	0.06
	HippoR	4	1.90	3.22	2.46	0.59
	RatioHippRToBasPon	4	0.18	0.41	0.28	0.11
	Part 1	4	0.00	14.00	6.50	7.05
	Part 2	4	30.00	63.00	48.75	14.31
20-29	Age	15	20.00	28.00	23.13	2.20
	BasPon	15	5.50	9.95	8.42	1.13
	AmygL	15	0.77	1.44	1.16	0.17
	RatioAmygLtoBasPon	15	0.10	0.21	0.14	0.03
	HippoL	15	1.66	2.35	2.03	0.24
	RatioHippoLToBasPon	15	0.21	0.32	0.24	0.03
	AmygR	15	0.90	1.76	1.25	0.26
	RatioAmygRToBasPon	15	0.11	0.27	0.15	0.04
	HippoR	15	1.80	2.90	2.54	0.31
	RatioHippRToBasPon	15	0.22	0.35	0.30	0.04
	Part 1	15	0.00	20.00	6.33	5.96
	Part 2	15	33.00	68.00	46.20	9.33
30-39	Age	10	30.00	38.00	35.20	3.22
	BasPon	10	6.48	11.86	9.03	1.96
	AmygL	10	1.05	1.70	1.43	0.17
	RatioAmygLtoBasPon	10	0.11	0.22	0.17	0.04
	HippoL	10	1.33	2.77	2.17	0.46
	RatioHippoLToBasPon	10	0.19	0.33	0.25	0.05
	AmygR	10	0.93	1.86	1.42	0.34
	RatioAmygRToBasPon	10	0.10	0.25	0.16	0.05
	HippoR	10	1.56	3.78	2.50	0.73
	RatioHippRToBasPon	10	0.17	0.54	0.29	0.11
	Part 1	10	0.00	16.00	4.50	5.72
	Part 2	10	29.00	62.00	40.70	9.94
40-49	Age	18	40.00	49.00	44.22	3.23
	BasPon	18	6.50	15.14	10.13	1.94
	AmygL	18	0.66	2.42	1.27	0.41
	RatioAmygLtoBasPon	18	0.10	0.20	0.13	0.03
	HippoL	18	1.41	3.06	1.92	0.37

	RatioHippoLToBasPon	18	0.13	0.29	0.20	0.04
	AmygR	18	0.76	1.84	1.23	0.28
	RatioAmygRToBasPon	18	0.10	0.18	0.13	0.03
	HippoR	18	1.48	2.72	2.21	0.34
	RatioHippRToBasPon	18	0.16	0.31	0.22	0.04
	Part 1	18	0.00	17.00	3.78	4.75
	Part 2	18	22.00	60.00	40.83	9.29
50-59	Age	11	50.00	59.00	54.64	3.26
	BasPon	11	6.91	11.84	9.25	1.62
	AmygL	11	0.75	1.75	1.19	0.28
	RatioAmygLtoBasPon	11	0.10	0.23	0.14	0.04
	HippoL	11	1.36	2.57	1.93	0.37
	RatioHippoLToBasPon	11	0.16	0.25	0.21	0.04
	AmygR	11	1.05	2.33	1.43	0.38
	RatioAmygRToBasPon	11	0.10	0.23	0.16	0.04
	HippoR	11	1.55	2.98	2.16	0.46
	RatioHippRToBasPon	11	0.17	0.37	0.24	0.07
	Part 1	11	0.00	13.00	4.73	4.58
	Part 2	11	29.00	55.00	43.09	8.95
60-69	Age	10	61.00	69.00	64.80	2.10
	BasPon	10	6.48	13.33	9.98	2.05
	AmygL	10	1.06	1.78	1.33	0.23
	RatioAmygLtoBasPon	10	0.10	0.22	0.14	0.04
	HippoL	10	1.30	2.71	2.12	0.47
	RatioHippoLToBasPon	10	0.15	0.32	0.21	0.05
	AmygR	10	0.98	1.50	1.24	0.15
	RatioAmygRToBasPon	10	0.10	0.18	0.13	0.03
	HippoR	10	1.52	3.11	2.29	0.40
	RatioHippRToBasPon	10	0.16	0.35	0.24	0.06
	Part 1	10	0.00	8.00	3.30	2.31
	Part 2	10	32.00	49.00	41.30	5.10
70-79	Age	10	70.00	78.00	73.00	2.91
	BasPon	10	6.07	10.69	8.82	1.33
	AmygL	10	0.80	1.29	1.00	0.17
	RatioAmygLtoBasPon	10	0.10	0.14	0.12	0.02
	HippoL	10	1.27	2.38	1.64	0.30
	RatioHippoLToBasPon	10	0.14	0.29	0.19	0.04
	AmygR	10	0.75	1.85	1.20	0.35
	RatioAmygRToBasPon	10	0.10	0.20	0.14	0.04
	HippoR	10	1.71	3.11	2.07	0.42
	RatioHippRToBasPon	10	0.18	0.32	0.24	0.05
	Part 1	10	0.00	15.00	3.90	4.84
	Part 2	10	27.00	54.00	42.80	8.47
80-88	Age	2	81.00	82.00	81.50	0.71
	BasPon	2	5.67	9.80	7.74	2.92
	AmygL	2	0.93	1.29	1.11	0.25

	RatioAmygLtoBasPon	2	0.13	0.16	0.15	0.02
	HippoL	2	0.94	1.63	1.29	0.49
	RatioHippoLToBasPon	2	0.17	0.17	0.17	0.00
	AmygR	2	0.84	1.22	1.03	0.27
	RatioAmygRToBasPon	2	0.12	0.15	0.14	0.02
	HippoR	2	1.11	2.23	1.67	0.79
	RatioHippRToBasPon	2	0.20	0.23	0.22	0.02
	Part 1	2	4.00	15.00	9.50	7.78
	Part 2	2	34.00	49.00	41.50	10.61

Appendix 6: Turn-It-In Report

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