

The Hippocampus, Amygdala and their principal output tracts in Major Depressive Disorder

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Thesis submitted for the degree of

Doctor of Philosophy

University of Dublin, Trinity College

2019

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DECLARATION

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Dr Darren Roddy December 2018

SUMMARY

Major depressive disorder is a common, debilitating illness. Despite its high prevalence rate and global disease burden, the biological mechanisms underlying this disorder remain largely unknown. Evidence points towards the involvement of the limbic system deep within the brain. This system processes memory, emotion and arousal, all of which are affected by depression symptoms. Two key regions in the limbic system are the hippocampus and the amygdala.

The hippocampus is important for memory formation, with the left hippocampus particularly important for episodic memory. The previously well-documented changes in this structure in depression may account for the common global memory and cognition impairments found in this condition. The amygdala has been shown to be fundamental to the generation and recognition of emotional responses, with the right side demonstrating more involvement in negative emotional responses compared to the left side, which processes more positive emotions. Although evidence exists for a role of this structure in depression, this evidence is less extensive than that of the hippocampus. Both of these structures have widespread connections across the entire brain and each has a clearly defined major white matter bundle, or output tract, that connects each structure to its principal downstream effectors. These tracts are the fornix, which conveys processed memory information from the hippocampus, and the stria terminalis, which conducts emotionally-laden output from the amygdala. Both these tracts terminate in the basal forebrain and hypothalamus, regions important for pleasure and stress responses, respectively. There is limited evidence for the involvement of both of these tracts in depression.

Using advanced volumetric and diffusion neuroimaging methods, combined with novel bespoke analyses of these images, this study has found noteworthy differences in all four structures in a well-characterised group of eighty-three patients with depression compared to eighty controls. Hippocampal differences were found to be confined to the core processing areas, more so on the left. There was evidence of an

extension of pathology in patients with recurrent depression when compared to first presentation patients who exhibited more restrictive structural changes. The amygdala showed an exaggeration in the normal right-left volume imbalance, driven by enlargement of right stress-associated centromedial output areas. Through measurement of the cortisol awakening response in a subset of participants, the right amygdala revealed an association with abnormal stress responses in depression. Both the fornix and the stria terminalis showed localised differences along distinct sections of their tracts suggestive of abnormal axonal connectivity in depression. The aforementioned centromedial areas of the amygdala were found to be predictors of a depression diagnosis. These amygdala areas and a specific substructure within the hippocampus, the CA1 region, were also found to be predictors of disease duration in depression.

These findings reinforce a role for these four limbic regions in depression. The lateralised volume differences in each of these structures could account for many of the symptoms of depression, including low mood, anhedonia and cognitive disturbance found in the disorder. While these results are preliminary, they demonstrate the utility of my novel analyses by revealing deeper and more site-specific differences in depression. These findings place altered hippocampal and amygdala volumes at the centre of a limbic network influencing memory, emotion and arousal in depression. Further studies, refining these techniques and exploring the upstream and downstream components of these structures are already underway.

ACKNOWLEDGMENTS

Firstly, I would like to offer my sincere appreciation to TCD and the HRB for allowing me the opportunity to return to academic study after so many years. I have been blessed with a wonderful opportunity to invest time in an endeavour I truly enjoy. I would also like to thank my colleagues at TCIN for their support during my time there.

Specifically, I need to thank the following people who have helped me on this journey.

Dr Erik O'Hanlon

The computer wizardry and statistical witchcraft you conjured up on a daily basis felt almost like sorcery at times. However, the daily fancy coffees, ring doughnuts, laughs and genuine friendship were the true magic.

Professor Veronica O'Keane

Thank you for taking me under your wing and imparting a deeper wisdom and objectiveness than I would have ever discovered myself. I particularly appreciate you allowing me the freedom to express myself academically and for investing your time to make my research the very best it could possibly be. Your good cheer and endless grace allowed every obstacle to be overcome.

My parents

I thank both of you for putting up with yet another crazy career deviation! I'm sure this will be the last! For always sacrificing so much, and putting me first, and encouraging my love of learning I am deeply grateful. Betty and Harry, thank you for getting me where I want to be.

To Lauren and Penelope, my morning stars...

....I love you both.

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LIST OF ABBREVIATIONS

ACh: acetylcholine

AD: axial diffusivity

ANOVA: analysis of variance

ANCOVA: analysis of co variance

CA: cornu ammonis

CAR: cortisol awakening response

CRH: corticotrophin releasing hormone

CSD: constrained spherical deconvolution

CSF: cerebrospinal fluid

DWI: diffusion weighted imaging

EC: entorhinal cortex

EPI: echo planar imaging

FA: fractional anisotropy

FLAIR: fluid attenuated inversion recovery

FOD: fibre orientation distribution

FOV: field of view

FPD: first presentation depression

HARDI: high angular radial diffusion imaging

HE: hippocampus extended

HF: hippocampal formation

HP: hippocampus proper

HPA: hypothalamic pituitary adrenal

ILF: inferior longitudinal fasciculus

MD: mean diffusivity

MDD: major depressive disorder

MRI: magnetic resonance imaging

RD: recurrent depression

RD: radial diffusivity

R-L: right-left

SLF: superior longitudinal fasciculus

T1: T1 relaxation

T2: T2 relaxation

TE: echo time

TR: repetition time

TCIN: Trinity College Institute of Neuroscience

TCD: Trinity College Dublin

UCD: University College Dublin

YLD: years lived with disability

CHAPTER 1

INTRODUCTION:

LOW MOOD AND LIMBIC MATTERS

"Begin at the beginning and go on till you come to the end; then stop"

Lewis Carroll

Alice in Wonderland, 1865

1.1. MAJOR DEPRESSIVE DISORDER

Major depressive disorder (MDD)¹ is one of the most common psychiatric conditions. It causes significant morbidity and mortality worldwide and is predicted to be the leading cause of disability by 2030 (Lépine and Briley, 2011). The lifetime prevalence of MDD is 19.2%, and the yearly prevalence is 8.3% (Kessler et al., 2010), with the lifetime prevalence of the disorder predicted to increase to approximately 41% over the next thirty years (Moffitt et al., 2010, Lépine and Briley, 2011). Depression can begin in childhood, although the mean age of onset falls between 24.8 and 34.8 years of age (Costello et al., 2003, Weissman et al., 1996). Women experience depression more frequently than men across all age groups (Kessler et al., 2010), although death by suicide is significantly higher in men (Schwartz-Lifshitz et al., 2012). Several sociodemographic factors are associated with a depression diagnosis. Poorer academic achievement (Riglin et al., 2014), unemployment (Kessler et al., 2003), and poverty (Inoue and Kawakami, 2010) are each associated with higher rates of depression.

Depression is a leading cause for years lived with disability (YLD) (Vos et al., 2012), significantly impacting on quality of life. The illness is frequently recurrent and chronic, with patients often having many episodes in their lifetime (Kessler et al., 1997). MDD is also a substantial socioeconomic burden to society (Steensma et al., 2016) and is estimated to cost over \$26 billion in the United States each year, contributing to economic losses due to unemployment (Greenberg et al., 2003). Having MDD also decreases life expectancy (Laursen et al., 2016) mostly due to death by natural causes, frequently cardiovascular events (Dhar and Barton, 2016). Suicide can be a serious consequence of having depression, with up to 7% of men and 1% of women with a lifetime history of depression dying by suicide (Schwartz-Lifshitz et al., 2012). This compares to a rate of suicide in the Irish general population of 0.008%

¹ Throughout this thesis, the terms Major Depressive Disorder (MDD) and depression will be used interchangeably as the clinical syndrome MDD.

(National Suicide Research Foundation [www.nsfr.ie]). Treatment of depression usually involves a combination of pharmacological, psychological and social treatments (Taylor et al., 2009) in keeping with Engel's concept of the biopsychosocial model of disease (Engel, 1977). However, despite increasing recognition of MDD by clinicians and engagement with treatment by patients (Marcus and Olfson, 2010), about 15% of patients fail to respond to both first and second line treatments (Sinyor et al., 2010).

DIAGNOSIS

Depression is a clinical diagnosis. This means that there are no biological investigations that can be used to definitively diagnose the disorder. There are two primary diagnostic classification systems used for the diagnosis of MDD; the Diagnostic and Statistical Manual (DSM-5) (American Psychiatric Association, 2013) and International Statistical Classification of Diseases and Related Health Problems, 10th edition (ICD-10) (World Health Organization, 1993). Although ICD-10 is used extensively in Ireland by clinicians, in this thesis the DSM-5 system for diagnosis will be used due to its better suitability as a research tool (Tyrer, 2014). MDD is characterised by the core symptoms of low mood and anhedonia (lack of pleasure or interest), along with a combination of other symptoms that persist for at least two weeks. DSM requires that patients meet 5 of the following 9 criteria to warrant a diagnosis of MDD, of which at least one must be number 1 or 2:

1. Depressed mood or irritable most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful).
2. Decreased interest or pleasure in most activities, most of each day
3. Significant weight change (5%) or change in appetite
4. Change in sleep: Insomnia or hypersomnia
5. Change in activity: Psychomotor agitation or retardation
6. Fatigue or loss of energy
7. Guilt/worthlessness: Feelings of worthlessness or inappropriate guilt

8. Concentration: diminished ability to think or concentrate
9. Suicidality: Thoughts of death or suicide, or has suicide plan

In summary, depression is common, resulting in significant socioeconomic burdens both to the individual and to society as a whole. However, morbidity and mortality remain high despite treatments. Understanding the causes of MDD may result in improved treatments of this often-chronic condition.

DEPRESSION HYPOTHESES

The exact aetiology of depression remains elusive. However, theories of depression can be broadly divided into two categories: psychological and biological theories. Indeed the treatment of depression often involves combining both psychological and biological treatments to maximise outcomes (Kendrick and Peveler, 2010). While psychological theories are fundamental to our understanding of the disease (Beck, 1967, Seligman, 1972, Freud, 1917) and psychological treatments have proven benefits (Hofmann et al., 2012, David et al., 2018, van Emmerik et al., 2018), this thesis will focus solely on the potential brain changes in MDD. Multiple complementary biological theories concerning the genesis of depression and depression symptoms exist. These include the following:

- Genetic determinants – specific genes are activated or deactivated in depression (Levinson, 2006, Flint and Kendler, 2014, Dunn et al., 2015).
- Monoamines – reduced serotonin, noradrenaline and dopamine as a cause of depression, while increase increasing these neurotransmitters can relieve depression (Delgado, 2000, Hindmarch, 2002, Schildkraut, 1965).
- Neurotrophic and neuroplastic factors – depression results from aberrant nerve growth and connections in certain areas (Serafini, 2012, Duman, 2004, Duman and Li, 2012).

- Inflammation – inflammatory proteins (cytokines) both as a cause and a consequence of depression (Miller and Raison, 2016, Raison et al., 2006, Bullmore, 2018).
- Glutamate receptors – enhanced glutamate neurotransmission causing excitotoxicity results in depression (Mathews et al., 2012, Sanacora et al., 2012, Niciu et al., 2014).
- Hypothalamic-pituitary-adrenal (HPA) axis – chronic overactivation of the stress sensitive HPA axis from early life adversity can cause depression (O'Keane et al., 2012, Pariante and Lightman, 2008).
- Circadian rhythm– chronic body clock and sleep disturbances can result in depression (Kronfeld-Schor and Einat, 2012, Germain and Kupfer, 2008).
- Neural circuits – dysconnectivity across neural circuits accounts for the symptoms associated with depression (Williams, 2016, Ressler and Mayberg, 2007, Pandya et al., 2012)

The biological causes of depression are mostly likely multifactorial, involving different combinations of the above mechanisms in different patients, and also dependent on the particular circumstances of the individual (Fried and Nesse, 2014). MDD is a highly heterogeneous entity, with opposing symptoms (e.g. insomnia versus hypersomnia, psychomotor agitation versus retardation) often conflated into one illness. It is unlikely that a distinct single illness called 'major depressive disorder' actually exists, but rather the term unites multiple pathophysiological disturbances under a single umbrella entity. This poses problems for the taxonomy and phenomenology of depression. A single unified diagnosis creates the illusion that a diagnosis of major depressive disorder represents an established neurobiological entity, and that 'all depression is the same'. This over-inclusivity is a recognised criticism of the two common diagnostic systems for depression, ICD-10 and DSM-5, although subtypes of depression such as atypical and melancholic go some way to providing a solution to these problems, (Horwitz and Wakefield, 2007, Thomason, 2014, Pickersgill, 2014). Both diagnostic systems describe symptom and experiential clusters with no consideration of the biological mechanisms. Diagnostic systems in

psychiatry have been found to be mostly reliable but serious questions have been raised concerning the validity of the diagnoses. Different clinicians commonly diagnose the same illness when given similar symptoms, but *what exactly* they are diagnosing is as yet of unknown brain aetiology (Lieblich et al., 2015, van Weel-Baumgarten et al., 2000). Using new developments in biomarker research, the neuroscience community is working towards establishing a more biologically grounded taxonomy for depression (Williams, 2016, Krishnan, 2008). The body of work in this thesis was undertaken in the hope of contributing to this effort by investigating strategic limbic differences between depressed patients and healthy controls.

1.2. THE LIMBIC SYSTEM

The limbic system is an interconnected set of brain structures involved in emotion, memory, behaviour and motivation. Unfortunately the term limbic system is somewhat undefined, but is generally meant to include brain regions found along the borders of the lateral ventricles when the brain is sliced in the midline sagittal plane (latin; *limbus* meaning **border** or **margin**) (Pessoa and Hof, 2015). Such structures include the hippocampus, amygdala, cingulate gyrus and septal areas. Often the prefrontal structures such as the medial prefrontal and orbitofrontal cortices are included as well as subcortical areas such as the hypothalamus, basal ganglia and midbrain areas are included (Rajmohan and Mohandas, 2007). First described by Pierre Paul Broca to describe structures only surrounding the pineal gland (Broca, 1878), the term was expanded in the 20th century by James Papez² who first proposed

² A portion of my work during my PhD involved reconstructing the white matter tracts of the Papez circuit using diffusion imaging; i.e. the mammillothalamic tract, the cingulum, the fornix and thalamocingulate tract. The findings of this work have been presented at Neuroscience Ireland (2016, 2017, 2018), Brain Research Day (Ireland) (2017, 2018) and The Anatomy Society meetings (2017, 2018). A paper: *Papez's forgotten tract: 80 years of unreconciled findings concerning the thalamocingulate tract* has been accepted by Frontiers in Neuroanatomy.

a circuit for memory and emotion involving the hippocampus, mamillary bodies, anterior thalamus and their interconnecting white matter tracts (Papez, 1937) (Figure 1.1.). The orbitofrontal cortex, insular regions and amygdala were included as having a modulating influence on the core emotional and memory circuitry by Paul Yakovlev (Yakovlev, 1948). Paul MacLean further developed and expanded the concept of the limbic system to include the cingulate and prefrontal cortices (MacLean, 1949, MacLean, 1952) and proposed a mechanism for emotion to drive physiological function. Indeed, the concept of a single uniform limbic system is probably outmoded, with some authors suggesting that multiple interacting systems such as a 'emotional' limbic system consisting of the orbitofrontal cortex, amygdala and extended amygdala and a more 'memory' limbic system involving the entorhinal cortex, hippocampus, posterior cingulate and anterior thalamus (Rolls, 2015, Catani et al., 2013).

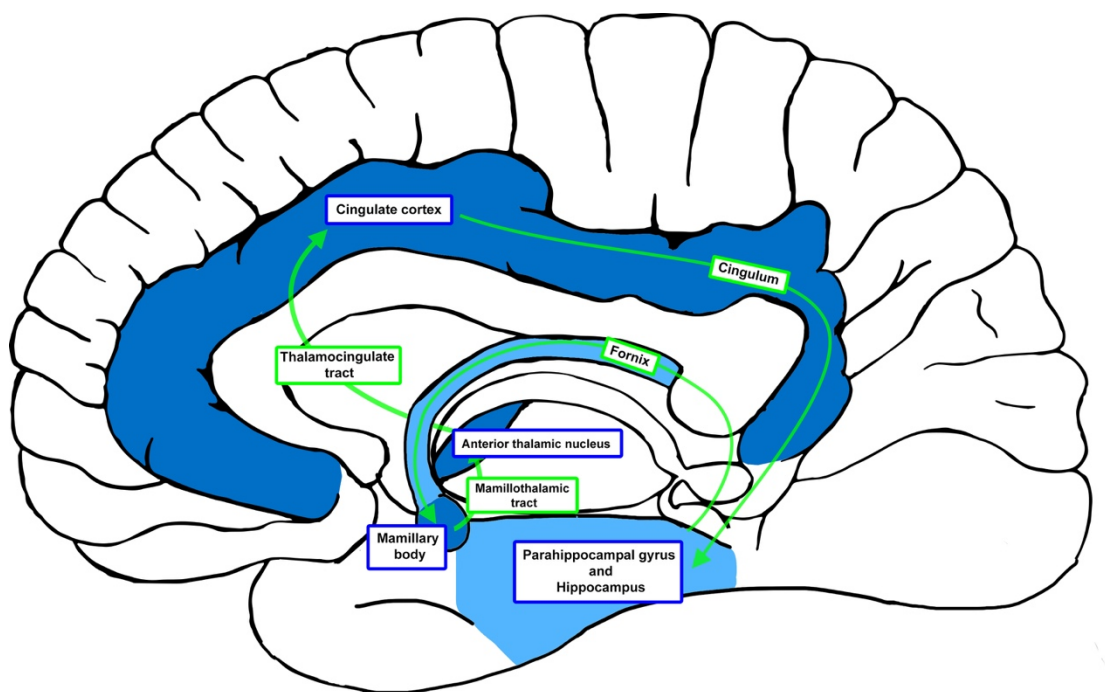


Figure 1.1. Papez circuit. The Papez circuit showing the grey matter (blue borders) and white matter tract (green borders). Taken from Weininger et al. 2018. Papez's forgotten tract: 80 years of unreconciled findings concerning the thalamocingulate tract (*Frontiers in Neuroanatomy*).

LIMBIC FUNCTION

The limbic system allows the brain to influence the endocrine system and autonomic system through the hypothalamus (Morgane et al., 2005, Pessoa and Hof, 2015, Lopes da Silva et al., 1990). It integrates the brain's pleasure and motivational centres (nucleus accumbens, septal areas), cognitive centres (orbitofrontal and prefrontal cortices), memory centres (hippocampus and prefrontal cortex), attentional centre (cingulate gyrus) and emotional centres (amygdala) to drive behavioural and physiological output (i.e. neuroendocrine and autonomic function). It is known to play a role in emotional processing (LaBar and Cabeza, 2006), motivation (Mogenson and Huang, 1973, Mogenson et al., 1980), learning and memory (Izquierdo et al., 1993, O'Mara, 2013), cognition (O'Keefe and Nadel, 1978), spatial awareness (O'Keefe and Dostrovsky, 1971) and social awareness (Frith, 1996). It has been shown to be involved in conditions such as in anxiety (Delvecchio et al., 2017, Rusch et al., 2001), schizophrenia (Abdul-Rahman et al., 2011, Simons et al., 2017, Smith et al., 2003), eating disorders (Schwartz et al., 2000, Val-Laillet et al., 2015), Parkinson's disease (Gyorfi et al., 2017), Alzheimer's disease (Christiansen et al., 2016), addictions (Kalivas and Volkow, 2005) and depression (2016, MacQueen and Frodl, 2011, O'Keane et al., 2012, Roddy et al., 2018a).

1.2.1. LIMBIC SYSTEM IN DEPRESSION

Many components of the limbic system have been shown to be involved in depression. The medial prefrontal cortex has been shown to exert more control over other parts of the brain in depressed compared to non-depressed groups. This is especially true for the posterior cingulate regions (Davey et al., 2017). The medial prefrontal cortex has been implicated in planning complex cognitive behaviour, personality, complex decision making and moderating social behaviours (Yang and Raine, 2009). It is particularly involved in the 'self-appraisal' processes. Overinhibition from the medial prefrontal cortex on the posterior cingulate has been suggested to account for the introspective ruminations characteristic of depression.

The medial orbitofrontal cortex has also been shown to be involved in depression, specifically associated with the expression and control of emotional states (Drevets, 2007). The stronger connections found in depression between the medial orbitofrontal cortex and the precuneus and angular gyrus regions (involved in the sense of self and attention/memory retrieval respectively) have been hypothesised to reflect the experiential symptom in MDD of reliving negative experiences. Connectivity complications from the basal forebrain award circuitry of adjacent to the orbitofrontal cortex (ventral striatum/nucleus accumbens areas) have been implicated in generating the anhedonic symptoms of depression (Heshmati and Russo, 2015). Deep brain stimulation of this area has been shown to alleviate depression symptoms (Bewernick et al., 2010).

Changes in the anterior and posterior cingulate cortices have been shown in depression, with anterior cingulate cortex dysfunction particularly associated with MDD. Grey matter neuronal and volume changes in depressed patients have been demonstrated in the subgenual part of the anterior cingulate (Ongur et al., 1998, Öngür et al., 2003). These findings correlate with the finding of increased subgenual anterior cingulate activity during sadness in healthy controls (George et al., 1995). Deep brain stimulation of the subgenual anterior cingulate has shown some success as a treatment for depression (Hamani et al., 2011, Lozano et al., 2008, Mayberg et al., 2016). Abnormal posterior cingulate connectivity has also been linked to MDD (Davey et al., 2017). While connectivity of this region appears to be increased overall (Zhou et al., 2010, Cheng et al., 2018), reduced integration of the posterior cingulate has been shown with the caudate regions (Bluhm et al., 2009). Patients who underwent successful deep brain stimulation treatment for depression showed increased metabolism and cerebral blood flow in the posterior cingulate (Leech and Sharp, 2013).

Two key hubs of the limbic system are the **hippocampus** and **amygdala**. The hippocampus is primarily involved in learning, memory and spatial awareness. The

amygdala assigns emotional salience to sensory inputs and drives emotional responses. Both structures are highly connected throughout the entire brain and are particularly integrated within the limbic system. The primary output tract from the hippocampus is the **fornix**, whereas as the primary output tract from the amygdala is the **stria terminalis**, closely aligned to the fornix. Combined, these structures allow contextually and emotionally-laden information to propagate throughout the limbic system and beyond, influencing an individual's behavioural responses. These four structures will be described in detail below and their investigation is the subject matter of this thesis. Evidence for the roles of these structures in depression will also be discussed below.

1.3. THE HIPPOCAMPUS

The Italian anatomist Arantius first described the characteristic appearance of human hippocampus in the 16th century and gave it the name hippocampus (greek; *hippókamos*, **seahorse**; from *ἵππος* [*hippos*], meaning **horse** and *κάμπος* [*kámpos*] meaning **sea monster**) (Schultz and Engelhardt, 2014, Andersen, 2007) (Figure 1.2). Other authors such as the Winslow highlighted the resemblance of the hippocampus to a ram's horn (Winslow, 1732), and Croissant de Garengot compared the hippocampus to the ancient Egyptian god Ammon's horn (de Garengot, 1742). The term *cornu ammonis*, for the true hippocampal core, was introduced by the Lorente de Nó (1934). The human hippocampus is a prominent C-shaped structure bulging in the floor of the temporal horn of the lateral ventricle (Andersen, 2007) and is a large elongated formation with a rostrocaudal extent of about 5 cm. Surgically, the hippocampus can be divided into a pre-commissural, supra-commissural, and retro-commissural sections depending on their relative positions to the corpus callosum (Blümcke et al., 2015). Whereas the first two parts are relatively small, the retro-commissural hippocampus represents the substantive portion of the hippocampal formation (Andersen, 2007).

The hippocampus is widest anteriorly where it bends inwards toward the medial surface of the brain. This region is known as the uncus. The number of rostrocaudal flexures in this bend varies substantially from individual to individual. Continuing caudally, the hippocampus tapers off and bends upward around the splenium of the corpus callosum. At the rostral limit of the lateral ventricle, the structure bends medially and then caudally. This caudal bend is made mostly by the dentate gyrus (Chapter 1.3.1.), whereas the medial bend is made by the subiculum (Chapter 1.3.3.). As the hippocampal formation curves dorsally to meet the retrosplenial region, the hippocampal cytoarchitecture becomes increasingly complex (Andersen, 2007). The hippocampus is not a single entity, but rather is made up of multiple interconnected substructures (O'Mara and Tsanov, 2015). These include the dentate gyrus, cornu ammonis (CA) regions, subicular and white matter regions. Unlike the amygdala (Chapter 1.4.), hippocampal subfields are very well defined and have been the subject of much research in both humans and particularly animals. The descriptions used below correspond to the output of the automated segmentation program used for this research, *FreeSurfer 6.0* (Figure 1.4.).



Figure 1.2. Hippocampus and seahorse. Hippocampus (left) and seahorse (right). Downloaded from <https://www.kidsdiscover.com/>.

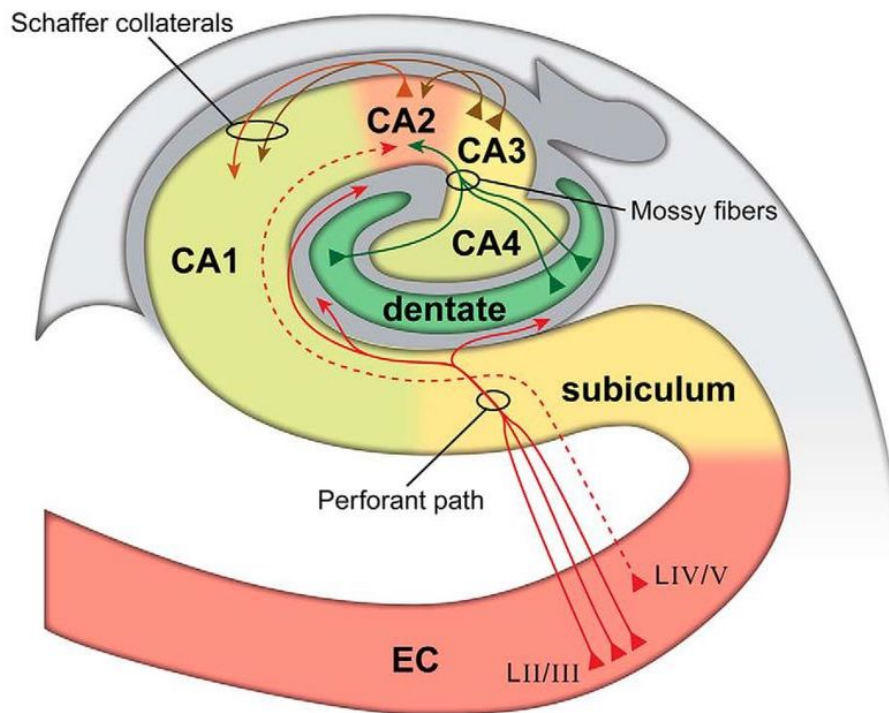


Figure 1.3. Diagram of intrahippocampal connections. CA, cornu ammonis; EC, entorhinal cortex; L, layer. Taken from Adamowicz *et al.* (2017).

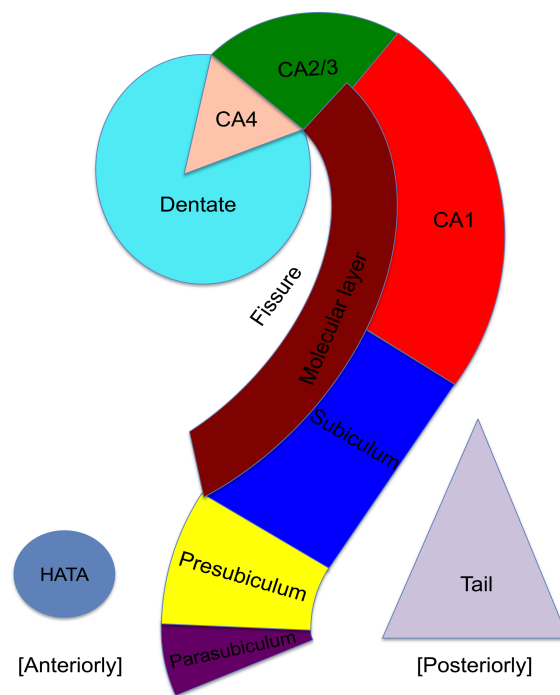


Figure 1.4. Schematic cross-sectional view of hippocampal substructures based on *FreeSurfer 6.0* output. The HATA and tail are not cross- sectional but placed either side for ease of viewing. CA, cornu ammonis; HATA, hippocampal amygdala transition area.

1.3.1. DENTATE GYRUS

The dentate gyrus (latin; *dentatus*, **tooth**) is the most medial part of the cerebral cortex. It begins about halfway rostrocaudally through the hippocampal head, forming a C-shaped structure. It is separated ventrally from CA1 and the subiculum by the hippocampal fissure. The dentate gyrus consists of three-layered allocortex that includes a sparsely populated molecular layer, a densely packed granular cell layer and polymorphic layer (Andersen, 2007). Containing only three layers, allocortex is considered the simplest and most primitive type of cortex (Insausti et al., 2017, Rose, 1927). The granular and molecular layers together constitute the dentate proper (*fascia dentata*) whereas the polymorphic layer is sometimes referred to as CA₄ (see Chapter 1.3.2.). The granule cell layer contains approximately 18×10^6 tightly-packed neurons (West et al., 2004) and their spiny dendrites branch exclusively into the molecular layer (Amaral et al., 2007). The granule cells also project basal dendrites into the polymorphic (CA₄) layer (Mrzljak, 1987). *FreeSurfer* automatic segmentation easily identifies the densely packed dentate granular cell layer. However, the molecular and polymorphic layers are redistributed together into the CA₄ region due to the inability to clearly distinguish the boundaries between them.

The dentate can be considered a key input region of the hippocampus (Aimone et al., 2011). Afferents originate in the superficial layers (layer II) of the entorhinal cortex and travel through the perforant pathway to synapse with the apical dendrites in the dentate molecular layer (Amaral et al., 2007, Andersen, 2007). The granule cells in turn send efferent output to the CA₃ region through the mossy fibre pathway. These two synapses are the first two components of the classic trisynaptic circuit (Figure 1.5.), probably most studied circuit of the entire nervous system in the past (Stepan et al., 2015), although it must be noted that this lamellar based circuit is neither as simple, nor probably as functionally fundamental as once thought (Moser, 2011). The mossy fibres also send off terminations to the polymorphic layer of the dentate (CA₄ region) (Andersen, 2007).

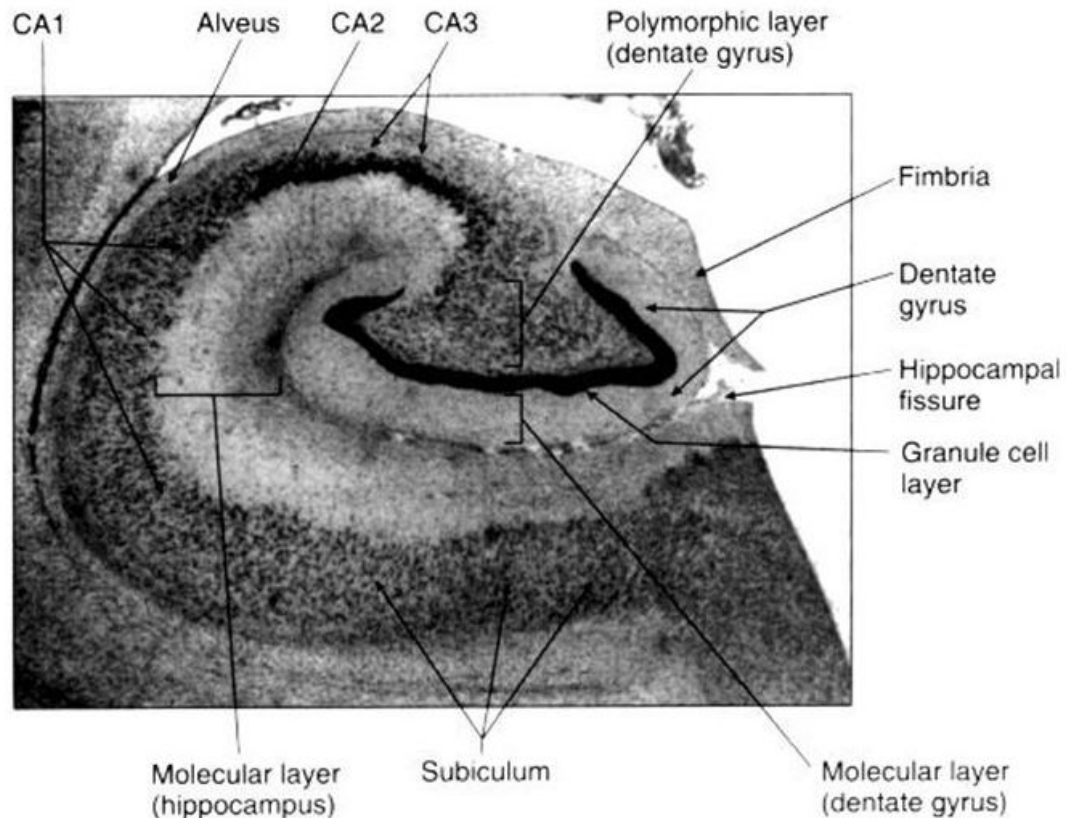


Figure 1.5. Cross section of human hippocampus. Note the 'swiss roll' arrangement of the structures. The granule cell layer of the dentate is particularly well demonstrated as a thick black line. CA, cornu ammonis. Image courtesy of Dr C. Wiegand.

NEUROGENESIS

The dentate gyrus is one of only two areas where adult neurogenesis is known to occur- the other being the subventricular zone in the walls of the lateral ventricles (Ming and Song, 2005). Within the subgranular zone of the dentate lie neural stem cells that retain the ability to divide into new neurons. These new cells exclusively propagate along the mossy fibre pathway to synapse with the CA3 region (Ming and Song, 2005, Abrous et al., 2005, Aimone et al., 2011). Exercise (Van Praag et al., 2005, Bjørnebekk et al., 2005), environmental enrichment (Kempermann et al., 1997, Lazarov et al., 2005) and seizure activity (Parent et al., 2006) are known to increase dentate neurogenesis. In contrast, aging (Cameron and McKay, 1999), stress (Schoenfeld et al., 2017) and viral infections (Sharma et al., 2002) are known to decrease new neuron formation, possibly mediated through excessive HPA axis activity (Lehmann et al., 2013). Reduced neurogenesis may play a role in depressive disorders (Samuels and Hen, 2011) and may be a potential cause for the frequently

reproduced finding of reduced hippocampal volumes found in MDD (Schoenfeld et al., 2017, Serafini, 2012).

ROLE IN MEMORY

The highly integrated (through extensive entorhinal afferents) and plastic nature of the dentate gyrus reflect the unique function of these cells in memory (Amaral et al., 2007). Their distinct connectivity along the mossy fibre pathway (dentate → CA3) is thought to be particularly important for pattern separation and completion (Rolls, 2013), which is a fundamental ability to discriminate between memory inputs and apply context to memories (Yassa and Stark, 2011, Aimone et al., 2011) (see also Chapter 1.3.2. and Figure 1.6.). This important function of the dentate gyrus has been supported through functional MRI imaging (Bakker et al., 2008). Imaging of the dentate has also demonstrated its role in new memory formation in human studies (Baker et al., 2016), with the effects of age on dentate volume potentially mediating the adverse effects of age on memory (Dillon et al., 2017). A role for the dentate gyrus has been suggested in schizophrenia (Walton et al., 2012), bipolar affective disorder (Elvsåshagen et al., 2013), mild cognitive impairment (Yassa et al., 2010) and Alzheimer's disease (Bocchetta et al., 2018).

DENTATE GYRUS AND DEPRESSION

Post-mortem studies have found evidence for apoptosis (Lucassen et al., 2001) and reduced thickness (Stockmeier et al., 2004) in the dentate gyrus of depressed patients. It is also known that anti-depressants can cause neurogenesis and dendritic sprouting in the dentate (Hanson et al., 2011). A post-mortem study of depressed patients showed increased progenitor cells in the subgranular layer of the dentate in depressed patients who were taking antidepressant medication (Boldrini et al., 2009). Various structural neuroimaging studies also have found dentate volume reductions along with other substructure changes in depression (Huang et al., 2013, Han et al., 2016, Treadway et al., 2015) (see Chapter 1.3.6. and Table 1.1). For these reasons, a central role for this hippocampal substructure has been suggested in depression (Sahay et al., 2007).

1.3.2. CORNU AMMONIS

The term cornu ammonis was first formalized by Rafael Lorente de No, who also suggested the classic four fields (CA1-4) of the hippocampus proper, based on types of pyramidal cells and their location and connections (Lorente de Nó, 1934). Larger giant pyramidal cells within the hilum of the dentate and subjacent to the fimbriae were allocated to subfields CA₄ and CA₃ respectively, whereas the smaller pyramidal cells were allocated to subfields CA₂ and CA₁. CA₁₋₃ only are *true* cornu ammonis subfields, with the CA₄ subfield most often considered as functionally part of the dentate region (Amaral, 1978). All CA subfields are three-layered allocortex (Rose, 1927, Andersen, 1975, Andersen, 2007). The CA regions are true hippocampus: i.e. cells located in the hippocampal gyrus and not within the adjacent dentate and subicular regions, and has been termed the *hippocampus proper* (Andersen, 2007).

The most superficial layer is populated sparsely with neuronal bodies and mostly contains the apical dendrites of the underlying pyramidal cells, interneurons and glial supporting cells (Capogna, 2011). This layer can be histologically divided into *stratum lucidum* (CA₃ only), *stratum radiatum* and *stratum lacunosum-moleculare*. However, only a single darkly contrasted superficial layer can be seen on MRI, which is termed the *stratum moleculare* or molecular layer. The molecular layers of the CA₁₋₃ subfields and subiculum are impossible to distinguish from each other on MRI and are therefore segmented out as a single large molecular layer abutting the fissure and above the CA and subicular regions. The middle layer of the cornu ammonis is the *stratum pyramidale* or pyramidal layer. This contains the cell bodies of the principal excitatory neurons of the hippocampus. It also contains many interneurons (Andersen, 1975). The deepest layer is known as the *stratum oriens* and comprises the basal dendrites and interneurons. The *strata pyramidale* and *oriens* (without the superficial layer) are segmented together to compute each subfield during segmentation.

CA1

This is by far the largest of the four cornu ammonis regions with approximately 14×10^6 neurons (West et al., 1994). From a phylogenetic perspective it shows striking advancement in humans and non-human primates compared to older species, with the CA1 subfield being significantly larger and having more complex cytoarchitecture (Stephan, 1983). It is difficult to distinguish from the adjacent subiculum using segmentation and is computed approximately from where the hippocampus turns upwards. It is differentiated from the CA2/3 region on the opposite side due to the increased thickness of the CA1 region compared to CA2/3. It is the final component of the aforementioned trisynaptic circuit (Figure 1.5.) where the Schaffer collateral fibres from the CA3 subfield terminate. CA1 also receives distinct inputs from the branches of the perforant pathway from the superficial entorhinal cortex (layer III), called the temporoammonic pathway. This is also called the monosynaptic pathway to distinguish it from the trisynaptic inputs (Suh et al., 2011) (Figure 1.5.). CA1 has two dominant outputs, to the subiculum (where it in turn outputs to the deep entorhinal cortex V) and also directly to the deep entorhinal cortex.

ROLE IN MEMORY

The CA1 neurons are the target of the multiple CA3 Schaffer collaterals and also receive inputs directly from the perforant path. They are uniquely placed for the role of pattern completion (Rolls, 2013) - a function of memory that allows separate but associated memory inputs to be co-localised for efficient retrieval (Rolls, 2010). This ability has also been suggested to allow accurate generalization if a particular memory isn't immediately accessible (Rolls, 2016). The CA1 region is known to be involved in autobiographical memory (Kohler et al., 2015, Zola-Morgan et al., 1986, Bartsch et al., 2011) and contextual memory retrieval (Zola-Morgan et al., 1986, Chen et al., 2011). It may also have a role in autonoetic consciousness (Bartsch et al., 2011). Autonoetic consciousness is a form of self-awareness that allows us to mentally place ourselves in the past, present or future and therefore enables an individual to examine their own thoughts (Tulving, 1985). It is thought to be an important capacity in establishing a stable 'sense of self' (Sutin and Robins, 2008). CA1 pathology has

been shown in Alzheimer's disease (Mueller et al., 2007) and Lewy body dementia (Adamowicz et al., 2017).

CA1 AND DEPRESSION

The CA1 region has a particularly high expression of multiple 5-HT receptor subtypes and therefore may be especially sensitive to disruptions in the serotonergic system (Dale et al., 2016). Long term corticosteroid exposure, a model of MDD in rats, has been shown to lead to attenuated serotonin responses in the CA1 region (Karten et al., 1999). The CA1 is also the hippocampal subfield most vulnerable to excitotoxicity (Coultrap et al., 2005) and post-mortem studies show evidence of extensive CA1 neuronal apoptosis relative to non-depressed controls (Lucassen et al., 2001), as well as reduced CA1 thickness (Stockmeier et al., 2004). To date, no imaging study has found changes in the CA1 region in living depressed individuals. In fact, several studies finding changes in various hippocampal substructures (Cho et al., 2013, Gyorfi et al., 2017) have failed to demonstrate CA1 changes (see Chapter 1.3.6. and Table 1.1)

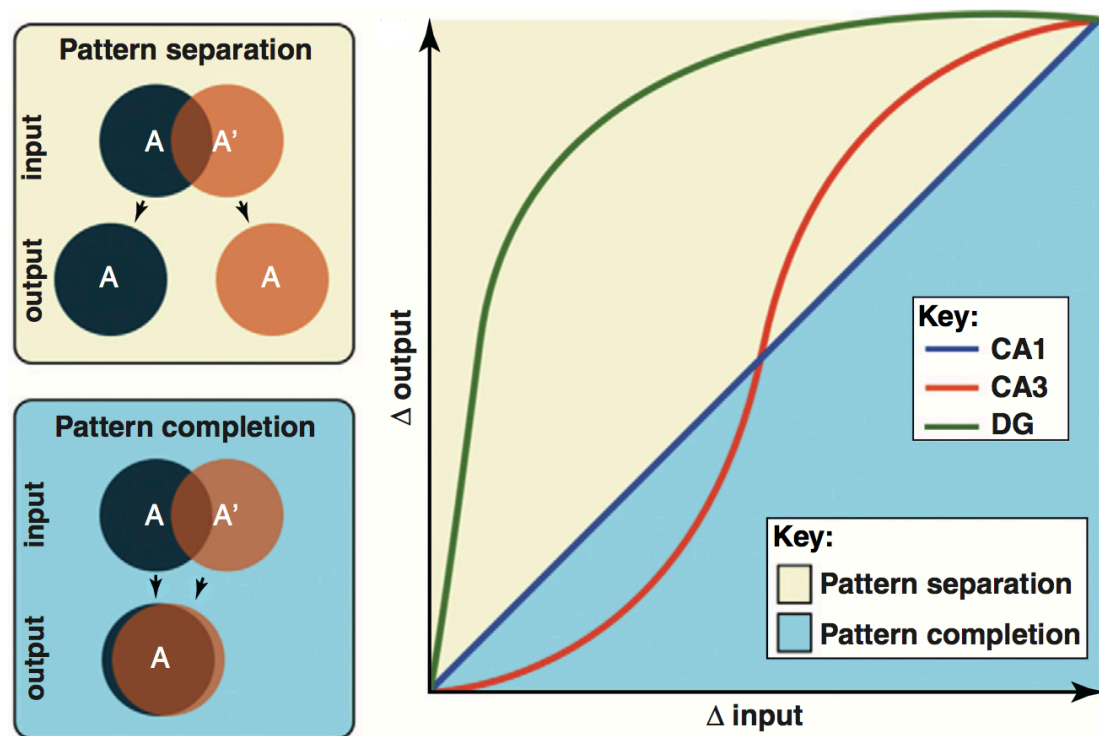


Figure 1.6. Pattern separation and completion in the hippocampus. Taken from Yassa and Stark (2011).

CA2

The CA2 is a relatively small and indistinct area interposed between the larger and more prominent CA1 and CA3 regions, with a relatively compact pyramidal layer (Andersen, 2007). It is difficult to distinguish histologically from the adjacent CA3 region and has been understudied compared to the larger subfields. Due to the inability to distinguish the CA2 from the adjacent CA3 region using MR imaging, and the similarity between the pyramidal layers of both subfields, *FreeSurfer* automatic segmentation combines the two subfields into a single CA2/3 region.

CA2 IN DEPRESSION

Despite being indistinct histologically and radiographically from the CA3, the CA2 displays distinct connectivity, receiving perforant path input from layer II of the entorhinal cortex (Chevalleyre and Siegelbaum, 2010) and direct input from the amygdala (Pikkarainen et al., 1999). This small subfield also receives prominent hypothalamic input, including direct input from the CRH (corticotrophin releasing hormone) producing paraventricular nucleus (Zhang and Hernandez, 2013) and histamine input from the tuberomammillary nucleus (Cui et al., 2013). It has been suggested to play a particular role in memory relating to socialisation (Hitti and Siegelbaum, 2014). This would be consistent with its unique connectivity with the amygdala and HPA axis, both implicated in socialisation. In keeping with its role in social cognition, CA2 pathology has been reliably found in schizophrenia (Narr et al., 2004, Benes et al., 1998). To date, little evidence exists for CA2 involvement in depression, however combined CA2/3 subfield volume loss was previously shown to be related to a depression diagnosis in Parkinson's Disease (Gyorfi et al., 2017) as well as in a unmedicated female patient cohort (Han et al., 2016).

CA₃

The CA₃ region lies between the CA₂ and CA₄ region. Due to the inability to distinguish CA₂ from CA₃ on neuroimaging (see above), automated segmentation combines the two subfields into a single CA_{2/3} substructure. CA_{2/3} continues until the hilum of the dentate. The combined CA_{2/3} region contains approximately 2.9×10^6 neurons (West et al., 1994).

CA₃ displays rich connectivity within the hippocampus. CA₃ is the termination of the mossy fibres originating from the granule cells of the dentate. These fibres end in the *stratum lucidum*. It also receives input from the superficial layers (II) of the entorhinal cortex along the perforant pathway. These fibres terminate in the *stratum moleculare*. CA₃ also collects afferents from the septal areas and commissural connections from the contralateral hippocampus. CA₃ outputs both forward and backwards along the path of hippocampal circuitry. However, axons from CA₃ overwhelmingly project forward to CA₂ and CA₁ through Schaffer collaterals. This is the final component of the trisynaptic circuit (Figure 1.5). CA₃ also back projects to the dentate gyrus as well as forming multiple recurrent connections within the CA₃ itself. As well as receiving inputs, CA₃ also outputs to the septal areas (Alonso and Köhler, 1982). These areas are important in pleasure and reward.

CA₃ AS A PACEMAKER?

This highly integrated subfield has been considered the pacemaker of the hippocampus, generating coordinated outbursts of cell assemblies such as theta and sharp waves that synchronize the activity of the entire brain (Artemenko, 1972, Wilson and McNaughton, 1994). These waves are associated with behaviours such as alertness and sleep respectively. Theta waves appear to represent an “online” hippocampal state critical for encoding and decoding of information (Buzsáki, 2002), whereas sharp waves may be important for reactivation of memories for consolidation to the cortex during sleep (Wang and Ikemoto, 2016).

ROLE IN MEMORY

As the destination of the mossy fibres from the dentate granular cells, CA₃ is also involved in the pattern separation and completion process of memory (Rolls, 2013). The CA₃ region has been shown to be involved in encoding spatial representations (O'Keefe and Nadel, 1978, Zhang et al., 2016), episodic sequence memory (Farovik et al., 2010) as well as memory retrieval (Chen et al., 2011, Schapiro et al., 2012). This hippocampal subfield has demonstrated a role in mild cognitive impairment (Yassa et al., 2010), but less so in Alzheimer's disease (Mueller et al., 2007). There is also evidence for aberrant neuronal activity in CA₃ in schizophrenia (Behrendt, 2010).

CA₃ AND DEPRESSION

CA₃ (combined CA_{2/3}) volume was found to be reduced in Parkinson's patients with comorbid depression. These volumes normalized following L-DOPA treatment (Gyorfi et al., 2017). Similar reductions were found in CA_{2/3} subfields in an unmedicated MDD female only cohort (Han et al., 2016).

CA₄

CA₄ was originally described as part of the cornu ammonis (Lorente de Nó, 1934). However, it is more often considered functionally part of the dentate gyrus (Amaral, 1978, Andersen, 2007). This is because it lies deep within the hilum of the dentate gyrus and all the subfield outputs project back to the dentate (Laurberg and Sørensen, 1981). It can be considered the polymorphic layer of the dentate. It contains approximately 1.7×10^6 neurons (West et al., 1994). Inputs are mostly mossy fibres originating in the dentate proper and a small contribution from CA₃ subfield. Automatic segmentation uses the entrance of the dentate hilum to delineate the transition between CA₃ and CA₄ subfields. The molecular layer of the dentate is indistinguishable from CA₄ and is included in this subfield. Therefore, using automated segmentation, the so-termed CA₄ region is in practice the combined polymorphic and molecular layers of the dentate.

1.3.3. THE SUBICULAR COMPLEX

The subicular complex (latin; *subiculum*, **support**) is the most inferior field of the hippocampal region and consists of several cortical components located between subfield CA1 of the hippocampus proper and the entorhinal cortex (Amaral and Witter, 1989). It can be divided into three subdivisions based on architectonics; the subiculum, presubiculum and parasubiculum (Witter et al., 1989). Different authors have suggested further divisions such as prosubiculum (Lorente de Nó, 1934), retrosubicularis (Brodmann, 1909) and postsubiculum (van Groen and Wyss, 1990), however, these subdivisions are inconsistently identified in primates (Ding, 2013). Therefore, only the consistent divisions found in humans are considered here; i.e. the subiculum, presubiculum and parasubiculum.

There has been some debate regarding whether the subiculum, presubiculum and parasubiculum should be considered as an anatomico-functional unit (Lopes da Silva et al., 1990, O'Mara et al., 2001). However, based on cytoarchitectonic and hodological considerations, one interpretation could be that they are discrete units. At the very least, the subiculum is a three layered allocortex and the pre- and parasubiculum are transitional multilaminar periallocortex (Andersen, 2007). Cytoarchitectonic differences also exist between the pre and parasubiculum. From a hodological perspective, the subiculum receives its major input from the CA1 subfields (Witter and Groenewegen, 1990, Amaral et al., 1991), whereas the pre and parasubiculum receive little hippocampal input, but rather its afferents are derived directly from cortical structures, similar to those of the entorhinal cortex (O'Mara et al., 2001). Subiculum efferents project hippocampal output to the deeper layers (V) of the entorhinal cortex (Witter et al., 2017), whereas the pre and parasubiculum projections are similar to sensory systems in that they project to the superficial entorhinal layers (Insausti et al., 2017). From here, information can join the hippocampal input pathways (perforant path). Another unique feature of the pre- and para-subiculum is a strong association with the anterior thalamus, allowing these nuclei to modulate hippocampal input (Amaral and Witter, 1989). As the subiculum is the output circuit of the hippocampus, it can be considered part of the greater

hippocampal formation whereas the pre- and para-subiculum may be specialised input hubs of the entorhinal cortex.

SUBICULUM

The subiculum is three layered allocortex consisting of a molecular, pyramidal cell and polymorphic layer, similar to the cornu ammonis fields (Andersen, 2007). It contains approximately 6×10^6 neurons (West et al., 1994) and lies between the CA1 and the presubiculum. The upper layer is continuous with the molecular layer of CA1 (*strata lacunosum-moleculare*) and it is impossible to distinguish between them on MRI. Thus, this layer is segmented along with the molecular layer of CA2/3 into a combined 'molecular layer' group. The well-defined enlarged pyramidal layer of the subiculum is distinct from the adjacent ill-defined cell layer of the presubiculum (see below) and this is used to delineate these two structures. However, the difference between the subiculum and CA1 is difficult to see on MRI, and the subiculum/CA1 boundary is approximated where the hippocampus turns upward.

The subiculum is the main grey matter output region of the hippocampus (O'Mara et al., 2001), with many reciprocal connections with diverse cortical areas (amygdala, prefrontal and entorhinal cortex) and subcortical areas (including the hypothalamus, septal nuclei and anteriomedial and anteroventral thalamic nuclei). It is highly connected with areas involved with cognition, reward, motivation, memory and emotional processing. However, the main subicular *afferent* is from CA1 and the main subicular *efferent* is towards the deeper layers (layer V) of the entorhinal cortex. There is suggestion of an anterior-posterior segregation of function within the subiculum, based on hodological considerations (O'Mara, 2005). The anterior component appears to have a role in HPA axis inhibition (Lowry, 2002), whereas the posterior component may be more involved with memory and spatial processing (Commins et al., 2002). This corresponds with theories on global hippocampal function being divided along the antero-posterior axis into 'emotional' and 'cognitive' components respectively (Poppenk et al., 2013, Fanselow and Dong, 2010). The subiculum has been shown to be involved in the pathology of Alzheimer's disease (Mueller et al.,

2007), and atrophy of the posterior subiculum is associated with memory impairment and tau and amyloid pathology in non-demented individuals (Lindberg et al., 2017).

SUBICULUM, HPA AXIS AND DEPRESSION

The subiculum has been suggested to be involved in HPA axis inhibition (O'Mara, 2005). A key target of the output from the anterior subiculum is the paraventricular nucleus (PVN) of the hypothalamus (Lowry, 2002, Risold et al., 1997). This nucleus contains parvocellular neurosecretory neurons that project to the median eminence, which secrete corticotrophin-releasing hormone (CRH) directly into the hypophyseal portal blood system (Malenka et al., 2009). CRH is carried to the anterior pituitary and stimulates the synthesis and release of adrenocorticotrophic hormone (ACTH), which in turn induces the release of cortisol from the zona fasciculata of adrenal cortex. Overactivity of the HPA axis occurs in depression and is generally accepted as being involved in the pathogenesis of at least some types of MDD (O'Keane et al., 2012, Dinan and Scott, 2005). Inhibition of the HPA axis is thought to be mediated by inhibitory GABAergic pathways that project from the anterior subiculum output to the PVN (Jacobson and Sapolsky, 1991, Herman et al., 1998, Herman et al., 1995). The anterior subiculum also selectively receives serotonergic neurons from the midbrain median raphe nuclei, allowing serotonergic tone to directly influence subicular output (Herman et al., 2002, Vertes and Hoover, 2008). As serotonin is a key neurotransmitter thought to be involved in depression, the subiculum may be a fundamental location for the interaction of serotonin and the HPA axis in depression. The subiculum has been shown to be smaller in some MRI studies in depression (Han et al., 2016, Wisse et al., 2015) (see Table 1.1).

PRESUBICULUM AND PARASUBICULUM

These areas lie between the subiculum and the entorhinal cortex and may be considered part of the parahippocampal region rather than the hippocampal region (van Strien et al., 2009). The presubiculum continues into the parasubiculum towards the midline and the parasubiculum occupies the most ventral edge (or shoulder) of the hippocampal fissure. In general, the parasubiculum parallels the presubiculum for

most of its course rostrocaudally, although it starts a little more caudally, and as such is slightly shorter than the presubiculum. Both structures are continuous with the retrosplenial cortex posteriorly, with suggestions that the pre/parasubiculum and the retrosplenial cortex may form an anatomico-functional unit (Goldman-Rakic et al., 1984, Barbas and Blatt, 1995, Berger et al., 1997).

As the cortex gradually changes from allocortical subiculum, through the transitional presubiculum, parasubiculum and entorhinal cortex towards isocortical perirhinal and posterior parahippocampal cortex, the cortical layers increase from three layers to six (Andersen, 2007) (Insausti et al., 2017). The presubiculum and parasubiculum (and entorhinal cortex) can thus be considered periallocortex, or intermediate cortex, with somewhat ill-defined layers. Common to all components of the periallocortex is the presence of a cell free zone, the *lamina dissecans* that dissects the cortex into approximately equal halves. This noticeably divided cortex has been known as 'schizo-cortex' (Rose, 1927) in the past, with the overall laminar organization of the parasubiculum similar to that of the presubiculum but with increasing complexity.

The presubiculum was assigned as area 27 by Brodmann (Brodmann, 1909). It contains a thickly myelinated molecular layer and a lamina *cellularis superficialis* (superficial cellular layer) containing rounded aggregated pyramidal neurons (Ding, 2013) above the *lamina dissecans* and a lamina *cellularis profunda* (deep cellular layer) below (Braak, 2012). The aggregated clumps of cells in the lamina *cellularis superficialis*, known as the presubicular clouds (Green and Mesulam, 1988) provides a distinctive heterogeneous contrast for *FreeSurfer 6.0*'s automated segmentation.

In the parasubiculum (Brodmann area 49), however, up to five or six layers may be recognizable histologically (Insausti and Amaral, 2012). In contrast to the presubiculum, its upper molecular layer is relatively indistinct (Insausti et al., 2017). Two neuronal layers can be seen between the molecular layer and the *lamina dissecans* and one layer is visible below. The *lamina dissecans* is also more ill-defined

in the parasubiculum relative to the presubiculum. Despite its clear histological layers, this substructure displays less lamination on MRI than either the adjacent presubiculum or entorhinal cortex. This drop in MR lamination between two readily identifiable laminated structures allows for ease of segmentation of the parasubiculum.

The major projection from the presubiculum is to the layer 3 of the entorhinal cortex and the major projection from the parasubiculum is to layer 2 of the same cortex (Andersen, 2007). Both these substructures also contain an excess of specialised grid (Boccaro et al., 2010), border (Lever et al., 2009) and head direction cells (Robertson et al., 1999), all of which are involved in aspects of spatial processing. Both structures are the primary targets of the parieto-medial-temporal pathway, which connects the caudal inferior parietal lobule, posterior cingulate cortex and retrosplenial cortex directly to the pre and para subiculum. This allows these parahippocampal regions to be involved in the processing of visiospatial information (Kravitz et al., 2011). It has been suggested that both these areas are neuroanatomically determined to facilitate processing of real and imagined scenes and scenic memories (Dalton and Maguire, 2017).

1.3.4. OTHER HIPPOCAMPAL STRUCTURES

ALVEUS AND FIMBRIAE

Throughout the majority of its rostrocaudal axis, the hippocampus is enveloped on its superior (dorsal) extent by white matter emanating from within the hippocampal formation. Efferent fibres from the hippocampus converge to form the alveus (latin; *alveus*, **channel** or **river**), which covers the ventricular surface of the hippocampus with a fine white matter coating. The alveus contains axons originating from all major areas of the hippocampus and dentate gyrus (Andersen, 2007). *FreeSurfer 6.0*

automatic segmentation identifies the alveus along the superior aspect of the hippocampus from the amygdala anteriorly to the fimbria posteriorly, in the floor of the inferior horn of the lateral ventricle.

These fibres converge to form a more discrete band, known as the fimbria (latin; *fimbria*, **fringe**). The fimbria increases in volume along the rostrocaudal axis, as it gathers more fibres along its course. It curves dorsally and towards the midline, to solidify into the fornix – the major output tract of the hippocampus (see Chapter 1.5.). The fimbria is defined in *FreeSurfer 6.0* automatic segmentation as starting at the midpoint of the body of the hippocampus along its the rostrocaudal axis, and continuing until the fornix begins around the level of the hippocampal tail.

The alveus-fimbria-fornix complex forms the principal white matter tract from the hippocampus (CA1 and subiculum mostly) to the hypothalamus, mammillary bodies, septal/forebrain regions and anterior thalamus. Fimbria volume reduction has been found in bipolar type II patients (Elvsåshagen et al., 2013), whereas no clinical study has identified alveolar change in any disorder. To date, no study has found changes in the alveus or fimbria in MDD.

HIPPOCAMPAL AMYGDALA TRANSITION AREA

Between the caudal end of the amygdala and the CA1 subfield, a transitional thin strip of grey matter called the Hippocampal Amygdala Transition Area (HATA) exists (Fudge and Becoats, 2012). This transitional area has distinct connective and cellular properties such that it can be considered neither true hippocampus nor true amygdala. Functionally the HATA is thought to carry afferent and efferent signals between the hippocampus and caudal amygdala (Fudge and Becoats, 2012). It has also been suggested that collaterals travel to prefrontal and hypothalamic regions. There is evidence that the HATA, as would be expected, has roles in both memory, emotion, and chronic stress. Only one clinical study to date has investigated this

region and a relationship was found between post-traumatic stress disorder with depression symptoms and HATA volumes (Averill et al., 2017).

HIPPOCAMPAL FISSURE

The hippocampal fissure, also known as the hippocampal sulcus, is the groove or space separating the dentate from the cornu ammonis and subiculum. This cerebrospinal fluid filled region is wider in first-episode schizophrenia, with decreased total hippocampal volumes (Smith et al., 2003). Fissure size was also associated with poor educational achievement and anxiety-depression symptoms. To date, no study has found a relationship between hippocampal fissure size and depression.

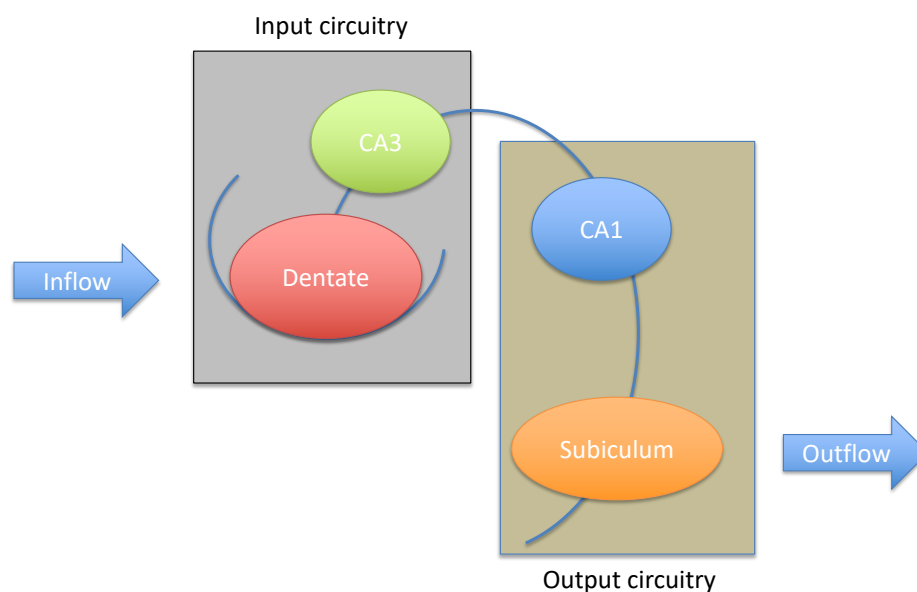


Figure 1.7. Schematic of hippocampal information flow. The main substructures are separated into input and output divisions. The hippocampus is divided into multiple anatomically distinct sub-regions; CA1-4, subiculum, and dentate gyrus. Other substructures and subdivisions of each structure exist but are not considered here for simplicity. Information enters the hippocampus from multiple cortical, subcortical and limbic areas mostly via the entorhinal cortex. This allows integration of cortically diverse information through the hippocampal input circuitry. This input circuitry is the location of the mossy fibre pathway, pattern separation/completion activity and where neurogenesis is known to occur. The output circuitry from the hippocampus is ultimately distributed (via the entorhinal cortex and fornix) to multiple cortical areas such as the orbitofrontal cortex, septal areas and anterior cingulate, important for mood, motivation and cognition. The CA1 and subiculum of the output circuitry are important in memory retrieval and hypothalamic-pituitary-adrenal axis inhibition.

1.3.5. WHOLE HIPPOCAMPAL VOLUMES IN DEPRESSION

Various meta-analyses using magnetic resonance imaging (MRI) to investigate the hippocampus as a sole structure (Cole et al., 2011, Videbech and Ravnkilde, 2004, McKinnon et al., 2009), or as part of a greater limbic system analysis (Koolschijn et al., 2009, Kempton et al., 2011, Arnone et al., 2012), have reported volume reductions of 4-10% in depression. Voxel-based morphometry measures also support the theory that hippocampal grey matter volume is lost in depression (Wise et al., 2016, Zhao et al., 2014, Du et al., 2012). Most evidence indicates that volume reduction is associated with a longer duration of depressive illness (i.e. MDD chronicity), a greater number of episodes or a cumulative depressive load (a combination of duration of episodes and number of episodes) of greater than two years (McKinnon et al., 2009). However, hippocampal changes have also been found in first episode patients (Frodl et al., 2002). Interestingly, a minority of studies have found that there are no differences in hippocampal volumetric measures between MDD and healthy control groups (Kempton et al., 2011, Dotson et al., 2009, Greenberg et al., 2008, Phillips et al., 2015).

Although the evidence indicates that there is global hippocampal volume reduction in MDD, inconsistencies across studies may be due to a number of factors. Depression is not a homogeneous clinical entity; with different patients often having different symptoms. Similarly, different grades of severity and subtypes of depression, state of relapse/remission, or different treatments, may be reflected in different changes in the hippocampus. Further variability may occur because the hippocampus consists of related substructures that combine organizationally to form the traditional functional unit (Abrous et al., 2005) (see also above). Definitions of what constitutes the hippocampus often lack consensus among researchers (Engelhardt, 2016) and may compromise inter-study comparison. For example, in a review of 423 hippocampal MRI studies, approximately 60 different anatomical guidelines for hippocampal volumes were used (Geuze et al., 2005).

1.3.6. HIPPOCAMPAL SUBFIELD SEGMENTATION IN DEPRESSION

A number of studies have found hippocampal substructure changes in depression. However, the data is somewhat conflicting with no clear pattern of substructural change emerging. This may be due to the small number of studies directly looking at this level of detail in depression (only seven published to date, excluding my recently released results) (see Table 1.1.). Discrepancies may also result from methodological inconsistencies between studies such as image acquisition differences and variations in segmentation protocols.

A study comparing 52 medication-free patients to 51 healthy controls found that the number of prior depressive episodes was correlated with reduced dentate volumes using *FreeSurfer 5.3* automated segmentation (Treadway et al., 2015). Using the same *FreeSurfer* version, another study found smaller left CA2/3, CA4, dentate and right subiculum volumes in 20 medication-free females compared to 21 aged matched controls (Han et al., 2016). A high field (4.7T) manual segmentation study showed smaller dentate volume in unmedicated patients (n=9) compared to medicated patients (n=11) or controls (n=27), as well as smaller total CA (1-3) regions in the hippocampal body of depressed patients (Huang et al., 2013). Depression in later life has also been associated with hippocampal substructure changes, with one study of thirty patients using *FreeSurfer 5.3* showing subiculum and CA2/3 changes (Lim et al., 2012) and a manual segmentation study of fifty patients showing CA1, CA3 and dentate changes (Choi et al., 2017).

However a recent study comparing hippocampal substructures across mood disorders (i.e. in both bipolar affective disorder and MDD) failed to find any significant changes in depression (Cao et al., 2017). When comparing 132 bipolar and 86 depressed patients to 152 controls using the latest version of *FreeSurfer (6.0)*, this study found that bipolar patients had reduced volumes across many hippocampal subfields, especially in the left CA4 and dentate and bilateral hippocampal tail. In particular they found that CA2/3, CA4 and tail showed negative correlations with the number of manic episodes. There was no relationship with depressive episodes in either MDD or bipolar affective disorder. The same group however found that three

weeks of electroconvulsive therapy (8 sessions) resulted in volume increases in 24 patients across CA, dentate and subiculum subfields (Cao et al., 2018). Another comparison study failed to find hippocampal substructure changes in MDD using an alternative algorithm (Automated Segmentation of Hippocampal Subfields) (Ota et al., 2017). In this study comparing 35 healthy controls, 20 schizophrenia patients and 26 depressed patients, only schizophrenia patients showed volume changes (reduced CA1 and dentate regions). A study using very high field (7T) manually segmented measures did not find differences in hippocampal subfields between 13 patients and 43 controls. However, multiple episodes of depression were associated with smaller subiculum volumes (Wisse et al., 2015).

Study	Protocol	No. of MDD	No. of Controls	Findings in MDD
(Lim et al., 2012)	Freesurfer 5.3	30	30	Reduced bilateral subiculum and CA2/3
(Han et al., 2016)	Freesurfer 5.3	20	21	Reduced dentate volumes in unmedicated, reduced CA1-3 in all MDD
(Wisse et al., 2015)	Manual	13	43	Reduced subiculum correlating with numbers of MDD episodes
(Treadway et al., 2015)	Freesurfer 5.3	52	51	Reduced dentate correlating with numbers of MDD episodes
(Choi et al., 2017)	Manual	50	50	Reduced bilateral CA1, CA3 and dentate
(Ota et al., 2017)	ASHS	26	35	No difference in MDD
(Cao et al., 2017)	Freesurfer 6.0	86	152	No difference in MDD

Table 1.1. Studies investigating substructural analysis of the hippocampus in MDD. All seven studies examining hippocampal substructures in controls v patients with MDD. Note the overall pattern of reduced subfields, but little consistency across subfields. CA, cornu ammonis, MDD, major depressive disorder. ASHS, automatic segmentation of hippocampal subfields.

Rather than specifically researching patients with MDD, some studies have investigated associations between depressive symptoms in other conditions and hippocampal substructure changes. A study of 35 newly diagnosed medication-free patients with Parkinson's disease without cognitive impairment showed a

relationship between CA2/3 volumes and depression scores (Gyorfi et al., 2017). These changes were normalized by L-DOPA administration, a standard treatment for Parkinson's disease. Similarly, depression scores were negatively correlated across multiple subfields; CA1, CA2/3, CA4, dentate, molecular layer and HATA volumes in patients with posttraumatic stress disorder (Averill et al., 2017). However, in a cohort of non-cognitively impaired older individuals, subthreshold depression symptoms were associated with less age related volumetric decreases in the right subiculum and CA1 than in those older individuals without depressive symptoms (Szymkowicz et al., 2017). The authors tentatively suggest that normal age-related atrophy of the hippocampus might be counteracted by depressive symptoms. However this proposition seems to go against the majority of current opinion in hippocampal research which suggests that depressive symptoms are associated with hippocampal shrinkage as opposed to being protective against hippocampal atrophy (See Chapter 1.3.5).

Adversity and stress have also been associated with hippocampal changes. In a cohort of 193 subjects aged between 18 and 25, childhood adversity and maltreatment was associated with left dentate, CA4 and CA2/3 changes with reductions of 63% and 6.1% respectively (Teicher et al., 2012). Associations were also found with maltreatment and left subiculum and presubiculum volumes. In a study of older adults, high levels of perceived chronic stress were also associated with smaller CA4-dentate and CA2/3 subfields (Zimmerman et al., 2016).

1.4. THE AMYGDALA

The amygdala (greek; *ἀμυγδαλή* [*amygdale*], **almond**), is an anatomically complex region of about 1500 mm³, comprising approximately 12 million neurons (Schumann and Amaral, 2005) lying within the mesiotemporal region, rostral and dorsal to the tip of the inferior horn of the lateral ventricle (Carpenter, 1985, Mai and Paxinos, 2011). It also lies just rostral and medial to the most anterior part of the hippocampal head (Figure 1.8.). This roughly almond shaped structure has proposed involvement in several psychiatric, behavioural and neurological disorders including depression (Hamilton et al., 2008, Young et al., 2018), bipolar disorder (Savitz et al., 2010, Foland-Ross et al., 2012), anxiety disorders (Wiest et al., 2006), drug-dependence (See et al., 2003), schizophrenia (Garcia-Marti et al., 2008), frontotemporal dementia (Boccardi et al., 2002), post-traumatic stress disorder (Morey et al., 2012) and Alzheimer's Disease (Yilmazer-Hanke and Hanke, 1999).

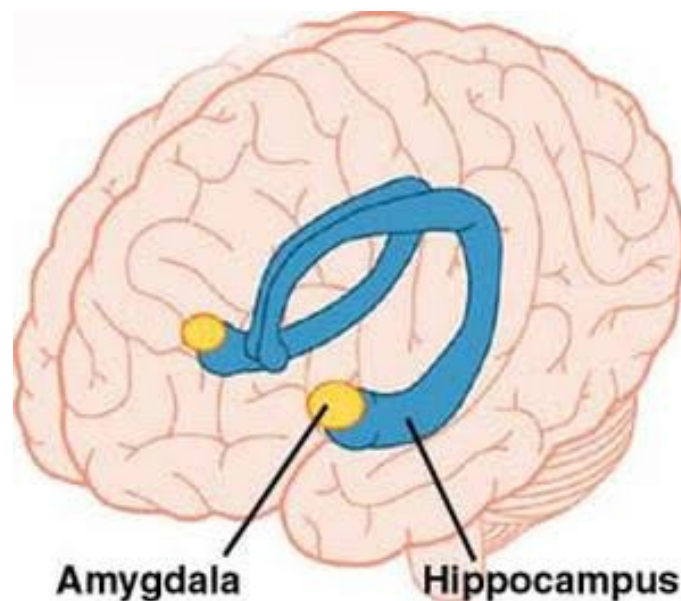


Figure 1.8. Position of amygdala relative to the hippocampus. Note the amygdala sits on top of the most anterior section of the hippocampus, and slightly medially. Also note that the hippocampus only continues back as the blue colour until the sharp bend posteriorly, from there onwards (i.e. the superior blue section in the anterior direction) is actually fornix. Taken from Quora.com.

The amygdala's widespread involvement in such assorted disorders is underpinned by its diverse functions, including adaptation to stress (Gray and Bingaman, 1996), sexual behavior (Everitt, 1990, Veening et al., 2005), olfaction (King, 2006) feeding (Bermúdez-Rattoni et al., 2004, King, 2006), arousal (McGaugh, 2006), memory (Roosendaal et al., 2006, Dalley and Everitt, 2009), reward (Hampton et al., 2007, Murray, 2007), emotional valence (Ball et al., 2009), bias (Sharot et al., 2007) and induction (Lanteaume et al., 2007), decision making (Floresco and Ghods-Sharifi, 2007, Bechara et al., 2003), fear conditioning, expression and extinction (LeDoux et al., 1988, Walker and Davis, 2002, Sotres-Bayon et al., 2006), and social behaviours (Schiller et al., 2009, Adolphs, 2009). Common among the different functions and pathology of the amygdala is its ability for multimodal information processing (LeDoux et al., 1990, Amaral et al., 2003, Linke et al., 1999, Aggleton and Saunders, 2000) and complex integration of both external and internal stimuli required for the modulation of higher cognitive functions and emotional behaviour. Functional studies in non-human primates show that only 4-7% of amygdalar neurons respond to unimodal stimuli of different types, whereas an overwhelming number of amygdalar neurons respond to multimodal or more complex sensory stimuli (like novelty, faces etc.) (Ono and Nishijo, 1992). Similarly, in humans, simple unimodal stimuli fail to elicit amygdala potentials, while stimuli with a larger cognitive/multimodal significance evoke sizeable amygdala potentials (Halgren, 1992). Therefore, it is its diversity of connections and multimodal inputs and outputs that allows the amygdala to be involved in such a high variety of functions. It has been suggested that a common role for the amygdala across all these functions is in generating an emotional 'tag' or 'label' in response to the environment resulting in subsequent motivational tags or labels. This allows the organism to respond in an emotionally appropriate manner to its environment (Geschwind, 1965, Aggleton, 1992).

Underpinning this functional and pathological diversity is the fact that this structure is not a solitary entity, but rather is made up of multiple related and interconnecting processing areas. The term amygdala was first coined by Burdach (Burdach, 1826),

(*German; mandelkerns = almond*) describing a unitary almond shaped grey matter mass in the mesiotemporal lobe. This initially only referred to the deep cortical-like nuclei of the amygdala. Subsequently, it was recognized that the amygdalar structure is more complex and consists of multiple heterogeneous nuclei (Völsch, 1906, Völsch, 1910, Kolliker, 1986). Later authors distinguished a more superficial 'corticomedial' group of nuclei from deeper nuclei. Indeed some early anatomists considered the central and medial amygdaloid nuclei as essentially extra-amygdalar nuclei and reclassified them as supra-amygdaleum (Brockhaus, 1939) or as part of the charmingly named substantia innominata (Hilpert, 1928).

The current situation in relation to the number of nuclei and subnuclei that constitute the amygdala varies among researchers (Sah et al., 2003). This may be due to the difficulty in separating the nuclei using conventional stains, the substantial overlap of the dendritic trees adjacent nuclear groups (Crosby and Humphrey, 1941, McDonald, 2003) and the differences of amygdalae across species. On the basis of cytoarchitectonics and comparison with experiments tracing neuronal connectivity in animals up to 24 separate nuclei have been recognised in the human amygdala (Mai and Paxinos, 2011). Most recent studies of individual amygdalar nuclei use variations of the nomenclatures proposed by Johnston (1923), Braak (1983) and Price (1987).

The current model of amygdalar organization suggests that it not a single functional or structural unit, but rather a fairly arbitrarily defined collection of adjacent cells in the temporal lobes based originally on gross anatomy and simple nucleic acid staining based histology (Petrovich et al., 2001, Swanson and Petrovich, 1998). While it is often treated as a unitary structure in neuroimaging studies (Abercrombie et al., 1998, Ashtari et al., 1999, Frodl et al., 2003), current opinion suggests that the amygdala is composed of heterogeneous nuclei, variously defined by their distinct cytoarchitecture (Price, 1987, de Olmos and Heimer, 1999), neurotransmitters (Sah et al., 2003) and connectivity patterns (Freese and Amaral, 2005, Freese and Amaral, 2009, Freese and Amaral, 2006, Alheid, 2003, Pitkanen, 2000, McDonald, 1998).

While the boundaries and nomenclature of amygdalar nuclei and their subnuclei change with species, investigative techniques and over time, most investigators use the nomenclature initially proposed by Johnston (Johnston, 1923) and further modified by Crosby and Humphrey (Crosby and Humphrey, 1941, Humphrey, 1968) and Price and Amaral (Price, 1987) and DeOlmos (de Olmos and Heimer, 1999). These nuclei can be easily visualised and discriminated using their cytoarchitectonic and nuclear features through the use of classic stains such as cresyl violet and acetylcholinesterase (AChE). Other naming and grouping systems exist, such as the 'refined topography' approach (Swanson and Petrovich, 1998) which divides the amygdala into four functional systems based on potential embryological and connectivity commonalities. While these naming systems have many virtues, the Johnston nomenclature (nine nuclei distributed into three functional groups) best serves the output of the automated segmentation program, *FreeSurfer*, and will be referred to from now on (Figure 1.9.).

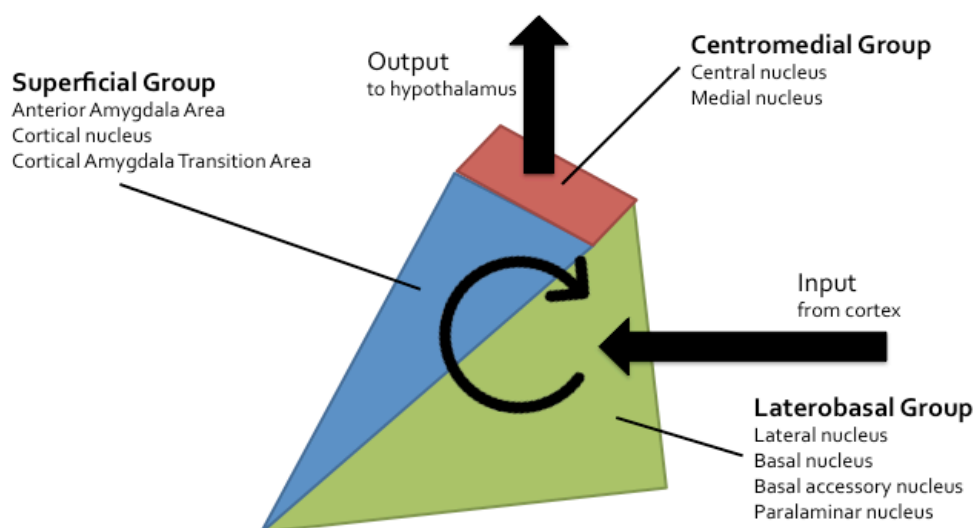


Figure 1.9. Amygdala substructures and composite groups. This schematic diagram shows the three basic functional amygdala nuclear groups are shown and their constituent nuclei. Also shown is a highly simplified input and output mechanism for the amygdala, displaying input through the laterobasal and output through the centromedial groups.

1.4.1. INDIVIDUAL NUCLEI AND GROUPS

Broadly speaking the amygdala can be divided into the superficial amygdala, the deep amygdala and the centromedial amygdala. The superficial amygdala is composed of the cortical nuclei (anterior and posterior), corticoamygdaloid transition area and the anterior amygdalar area. The superficial amygdala is 'allocortex', a fairly primitive 2-3 layered cortex that is highly conserved phylogenetically (Vogt, 1919). The entire superficial amygdala is generally associated with olfactory function, however inputs from other sensory modalities project here as well (Carmichael et al., 1994, Turner et al., 1980). There is some evidence that these superficial structures are specifically involved social functions in humans (Goossens et al., 2009), where they function to extract the social value of incoming sensory information and allocate appropriate emotional responses.

LATEROBASAL GROUP

The deep nuclei include the basal, accessory basal, lateral nuclei and paralaminar nuclei. Unlike other amygdaloid regions, this entire group of structures, otherwise known as the 'basolateral nuclear group', is remarkably progressive across the range of mammalian evolution, reaching its relatively greatest size in primates (Stephan, 1983). These nuclei are phylogenetically recent adaptations, and their development parallels the massive expansion of the temporal and frontal cortices in primates from which they receive their main inputs (Pabba, 2013). The lateral nucleus is the largest nucleus in most species, reaching its greatest extent in humans (Stephan and Andy, 1977, Pabba, 2013). It is the main input centre of the amygdala, and is the site where multimodal sensory information from visual and polysensory association cortices of the cortices project (Aggleton et al., 1980, Amaral and Insausti, 1992, Stefanacci and Amaral, 2000, Turner et al., 1980). These inputs are reciprocated back to the original cortical regions but can also be channelled into the basal and accessory basal nuclei, following a topography based on sensory modality (Pitkanen and Amaral, 1998).

Similar to the lateral nucleus, the basal nucleus also receives extrinsic projections from cortical areas, many of which it returns. The entorhinal cortex, insula and medial

and orbital prefrontal cortices project heavily to the basal nucleus (Amaral and Insausti, 1992, Carmichael et al., 1994, Carmichael and Price, 1995, Ghashghaei and Barbas, 2002, Van Hoesen et al., 1981). Hippocampal output from CA1 and subiculum subfields also send projections to terminate in the basal nucleus, specifically targeting the ventral basal nucleus and paralamina nucleus in the primate (Rosene and Van Hoesen, 1977, Saunders et al., 1988). The thalamus also directly projects to these nuclei (Carmichael and Price, 1995, Ghashghaei and Barbas, 2002, Mehler, 1980, Van Hoesen et al., 1981). The basal nucleus is comprised of three sub-regions; magnocellular, intermediate and parvocellular sub-regions, oriented dorsally to ventrally (Braak and Braak, 1983, Amaral and Bassett, 1989).

The accessory basal nucleus, located medial to the basal nucleus, receives many of the same intrinsic and extrinsic connections as the basal nucleus (Freese and Amaral, 2009). It is also divided into three sub-regions which have important differences embryologically, anatomically and hodologically; the magnocellular, parvocellular and sulcal divisions.

The paralamina nucleus lies wedged between the lateral, basal and accessory basal nuclei. It is also particularly well developed in primates and humans, such that is present in primates while almost non-existent in other species. It has an abundance of serotonergic receptors (Bauman and Amaral, 2005) as well as being exceptionally sensitive to corticotrophin releasing hormone (Sanchez et al., 1999). These properties give the paralamina nucleus a potential role in integration of mood and stress responses, and thus potentially a role in MDD.

CENTROMEDIAL GROUP

The central nucleus is the main recipient of efferent fibres from both the 'superficial' and 'deep' nuclei (Fudge and Tucker, 2009). This nucleus is the principal amygdala output to the hypothalamus via the stria terminalis and amygdalofugal pathway. The central nucleus is involved in HPA axis responses to systemic, i.e. physical stress (Dayas et al., 1999) and is considered one of the most peptide rich area of the brain (Gray and Magnuson, 1992)

This distinct nucleus is composed of striatal-like medium spiny neurons (Cassell et al., 1999, McDonald, 1982) and forms a continuum with the bed nucleus of the stria terminalis (BNST). The central nucleus is located dorsomedially in the rostral part of the amygdala, bordered laterally by the basolateral complex, dorsally by the globus pallidus, and medially by the stria terminalis (which is continuous with the central nucleus as it transmits its main output). This nucleus has four divisions: the capsular subdivision, lateral subdivision, intermediate subdivision, and medial subdivision (McDonald, 1982, Jolkkonen and Pitkanen, 1998).

The medial nucleus is found near the cortical surface and is bounded medially by the optic tract. It is visible along anteroposterior extend of amygdala medially and is often a slim and elongated structure. It also has four subdivisions: rostral, dorsocentral ventrocentral and caudal. Similar to the central nucleus, this nucleus also activates the HPA axis through the stria terminalis, but predominantly in response to psychogenic stressors rather than just physical stress (Jones et al., 2011, Myers et al., 2012). Of note, the medial nucleus also sends direct, as distinct from just via the stria terminalis, projections to the hypothalamic medial neuroendocrine region, particularly the paraventricular nucleus (Dayas et al., 1999, Petrovich et al., 2001).

Traditionally both the central and medial nuclei were historically pooled together as part of the superficial, more cortical group of nuclei, due to their position and

cytoarchitectonics. However, it has been suggested that the central and medial, nuclei have histochemical and developmental characteristics that are distinct from the cortical nuclei, and more similar to the nuclei found along their output pathway, the bed nucleus of the stria terminalis (de Olmos and Heimer, 1999).

The bed nucleus of the stria terminalis (BNST) (see also Chapter 1.6.) is almost identical to the central and medial nucleus cytoarchitecturally and histochemically, and both structures are linked by an almost continuous chain of small cellular islands that span the basal forebrain (McDonald, 1998). These structures together are known as the extended amygdala (Heimer et al., 1999). The scope of input and output of the extended amygdala have been determined predominantly in rodent species (Alheid, 2003) with few studies in the humans and primates. One diffusion connectivity study confirmed that the human BNST connects to amygdala, hypothalamic and forebrain regions (Kruger et al., 2015). The extended amygdala has been implicated in modulation of stress, fear and social responses and may have particular important in psychiatric disorders (Lebow and Chen, 2016), being implicated in depression (Ressler and Mayberg, 2007), aggression (Trainor et al., 2006), anxiety disorders (Ravinder et al., 2013), and post-traumatic stress disorder (Ressler et al., 2011).

In conclusion, amygdala nuclei can be functionally clustered into three groups- superficial, laterobasal and centromedial. The superficial group is a more primitive, evolutionarily conserved region and is related to olfactory and social function. The laterobasal is the largest group and is particularly enlarged in humans. This region receives, processes, returns and redistributes inputs from sensory and cortical regions. Processed outputs are sent both back to the reciprocal cortical areas from where they originated and are also transmitted to the centromedial group. The centromedial group forms the main output pathway of the amygdala. It is often considered as part of the extended amygdala, along with the aligned BNST, and is the primary locus through which the amygdala drives behavioural, autonomic and humoral responses via the hypothalamus.

1.4.2. AMYGDALA VOLUMES IN DEPRESSION

To date there have been no *FreeSurfer* investigations of the whole amygdala to date in MDD, as the amygdala segmentation module was only released last year (Saygin et al., 2017). However previous studies have investigated the whole amygdala in depression using manual segmentation techniques. Overall, the studies have been inconclusive, with some studies showing increased volumes in depression (Sandu et al., 2017, Lorenzetti et al., 2010), some studies showing decreased volumes (Zavorotnyy et al., 2018, Kronenberg et al., 2009) and some showing no change. Meta-analyses have confirmed these indecisive findings (Arnone et al., 2012, Hamilton et al., 2008), but treatment of depression (i.e. medications) may be correlated with increased volumes (Hamilton et al., 2008). The lack of consistent volumetric changes found in the amygdala in depression may be due to differences at the nuclear level being obscured by a lack of differences at the whole amygdala volume level.

1.4.3. AMYGDALA NUCLEI IN DEPRESSION

Very few studies have studied the individual amygdalar nuclei in depression. This is due to the inherent difficulty in defining the nuclei visually on MRI. Only one study has investigated this, finding a reduction in the laterobasal nuclei bilaterally in a group of twenty patients with recurrent depression compared to controls (Sheline et al., 1998). Manual segmentation was used in this study and the sample size could be considered relatively small. To date there have been no studies looking at the individual nuclei beyond the laterobasal nuclei (the largest and easiest to discern on MRI) in MDD.

1.5. THE FORNIX

The fornix (latin; *fornix*, **arch**) is the main output of the hippocampus. This C-shaped structure lies in the midline of the brain arising from the alveus on the dorsal surface of the hippocampus (Carpenter, 1991) (Figure 1.10.). Emerging from the alveus as the fimbriae (latin; *fimbria*, **fringe**) it travels caudally, curving superiorly and anteriorly under the splenium of the corpus callosum forming the crus (latin; *crus*, **leg**) of the fornix. Joining the crura are the crossing fibres known collectively as the hippocampal commissure (or dorsal hippocampal commissure); these fibres relay information between the two cerebral hemispheres³. The two crura then unite to form the body of the fornix. The fornix continues to arc anteriorly and ventrally where it becomes the columns of the fornix and splits into two discrete tracts at the level of the anterior commissure. About three-quarters of these fibres make up the post-commissural tract which originates from the subiculum of the hippocampus and pass behind the anterior commissure to innervate the mammillary bodies and hypothalamus (Carpenter, 1991). These fibres continue as the mammillothalamic tract to innervate the anterior thalamic nuclei as part of the Papez circuit (Papez, 1937, O'Mara, 2013). The remaining fibres pass in front of the anterior commissure, making up the pre-commissural tract. These fibres originate from the pyramidal cell layer of the hippocampus and innervate the septal area and basal forebrain (Thomas et al., 2011, Nolte, 2002, Yeo et al., 2013) Due to that fact that the fornix remains as two separate tracts except when united briefly at the body, it can be said that this structure is both a projection tract and a commissure (Thomas et al., 2011).

³ A portion of my work during my PhD involved supervising students (Anurag Nasa and Caoimhe Gaughan) investigating the dorsal hippocampal commissure. Using a similar technique used in this thesis to extract the stria terminalis, we used temporal region masks (temporal pole, amygdala, hippocampal and entorhinal volumes) to map the connectivity of the commissure. Interestingly most of the crossing fibres are not hippocampal in origin, but rather extrahippocampal. The findings of this work has been presented at Neuroscience Ireland (2018) and The Anatomy Society meeting, Oxford (2018). A paper: *The Dorsal Hippocampal Commissure; Untangling its connections* is being submitted to NeuroImage.

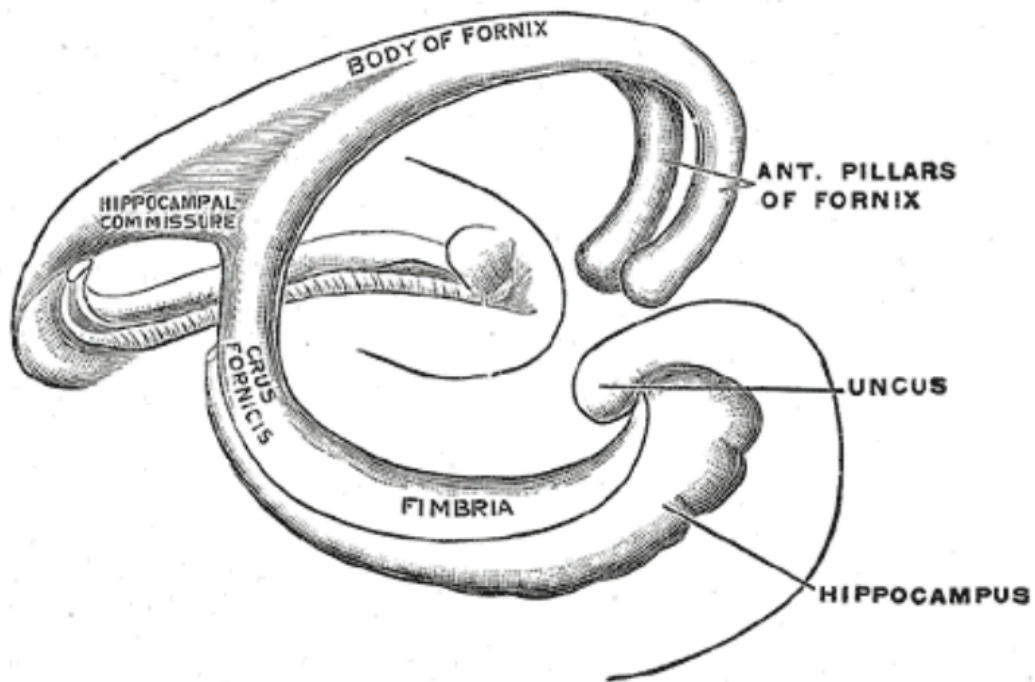


Figure 1.10. The fornix. Drawing of the fornix. Note it is a bilateral structure that unites macroscopically in the midline as the body of the fornix. The fimbriae is a continuation of the alveus along the dorsal aspect of the hippocampus. It can be clearly seen here that the fornix proper (as the crus) does not commence until the crus is travelling vertically – this is the same point used for our tractography dissection (see Chapter 2.7.1.). Taken from *Anatomy of the Human Body* by Henry Grey (Plate 747) (Gray and Lewis, 1918).

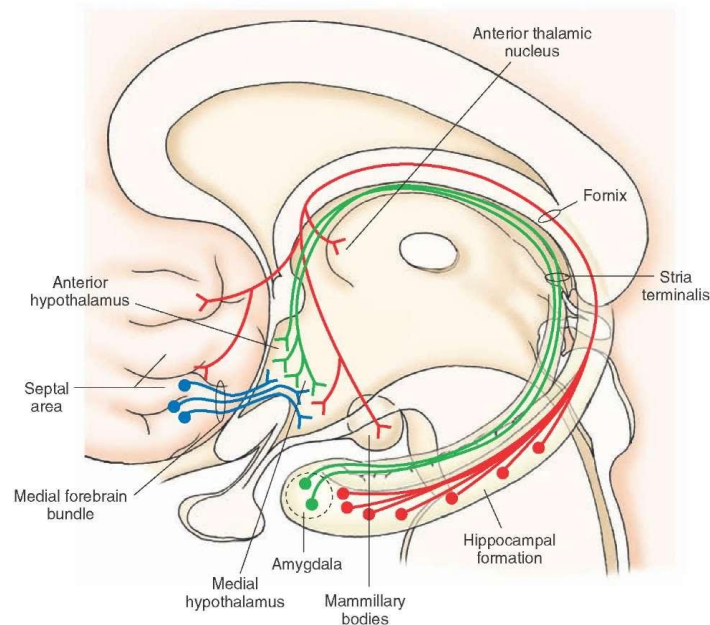


Figure 1.11. The stria terminalis and fornix together. Schematic illustration showing the connectivity of the fornix and stria terminalis. Note how the stria terminalis and fornix follow a closely aligned loop like path. Taken from [www. memorangapp.com](http://www.memorangapp.com).

The fornix is the white matter component of the limbic system which most notably has a role in memory (Nolte, 2002, Christiansen et al., 2016). Specifically, it forms an integral part of the classic Papez circuit (Papez, 1937). The fornix is thought to be involved in episodic memory functions through its post-commissural fibres as it connects structures from the extended hippocampal network to the mammillary bodies and forwards to the anterior thalamus and cingulum (Aggleton and Brown, 1999). Degradation of fornix fibres is associated with various degrees of memory loss (Aggleton and Brown, 1999, Christiansen et al., 2016, Thomas et al., 2011). In fact, the fornix has been shown to be negatively impacted in disorders such as Wernicke encephalopathy, multiple sclerosis, amnesia, mild cognitive impairment and some forms of dementia (Christiansen et al., 2016, Tang et al., 2017, Kern et al., 2012, Douet and Chang, 2014, Metzler-Baddeley et al., 2012, Gaffan and Gaffan, 1991). The pre-commissural fibres of the fornix project to the septal nuclei, nucleus accumbens and ventral striatum (Nolte, 2002). These areas are known to have a role in reward and reinforcement (Schultz, 1998, Nestler et al., 2002, Olds and Milner, 1954). It is suggested that these structures may mediate certain symptoms of depression including anxiety, reduced motivation and anhedonia (Nestler et al., 2002).

The fornix is clearly seen in standard T1 and T2 images of the brain as a solid white matter tract (Oishi et al., 2010). However, what is viewed as the fornix is actually the fornix-stria combination as it is difficult to distinguish the smaller stria terminalis from the fornix (Figure 1.11). Many studies have used diffusion-weighted imaging to investigate the fornix in health and disease (Thomas et al., 2011), however in most cases these studies are actually examining the fornix-stria combination, rather than the fornix alone. Decreased fractional anisotropy, as proxy indicators of disruption of white matter microstructure were found in the fornix of Alzheimer's patients (Tang et al., 2017), normal pressure hydrocephalus (Hattori et al., 2012) multiple sclerosis patients (Dineen et al., 2009) in comparison to healthy controls. Normal ageing is also known to negatively impact white matter tracts including the fornix (Jang et al., 2011, Metzler-Baddeley et al., 2011). Furthermore, diffusion imaging has implicated the fornix in a number of psychiatric disorders (Thomas et al., 2011), including bilaterally

reduced fractional anisotropy in schizophrenia compared to controls, suggesting a relationship between fornix degradation and schizophrenia (Fitzsimmons et al., 2009).

1.5.1. THE FORNIX IN DEPRESSION

The fornix is situated in the strategic position as the main output tract of the hippocampus, and because of the large body of evidence implicating the hippocampus in depression (Roddy et al., 2018a, Arnone et al., 2012, MacQueen and Frodl, 2011, Serafini, 2012, Videbech and Ravnkilde, 2004), alterations of the fornix white matter may also be found in MDD.

Depressive symptoms following traumatic brain injury have been associated with global changes in white matter, including the fornix (Raikes et al., 2018). Similarly, widespread white matter changes, again including the fornix regions, have also been seen in patients with MDD (Hollocks et al., 2015). However, past childhood adversity in otherwise healthy adults was not associated with any changes in the fornix (McCarthy-Jones et al., 2018), whereas a study investigating polymorphisms of the neuronal amino acid transporter SLC6A15 gene on the structural integrity of white matter tracts in MDD found correlations with a depressive diagnosis for multiple white matter tracts, but not the fornix (Choi et al., 2016). Changes in the aligned stria terminalis, but not the fornix, were found to be predictors of treatment outcomes in depression (Korgaonkar et al., 2014). Finally, a post-mortem study found no changes in fornical volume, diameter, length or lateralisation in patients with depression (Brisch et al., 2008). Similarly, no changes in myelinated axon density was found in the post-mortem fornix of deceased depressed subjects compared to controls or patients with schizophrenia suggesting a limited role for the structure in MDD (Williams et al., 2015).

1.6. THE STRIA TERMINALIS

The stria terminalis (latin; *stria*, **stripe**; *terminalis*, **terminal**) is the major output pathway of the amygdala (Lebow and Chen, 2016). It consists of a narrow band of white matter fibres that run along the lateral margin of the ventricular surface of the thalamus, tightly hugging the fornix and following its general loop like shape (Carpenter, 1991) (Figure 1.11.). It directly connects the output nuclei of the amygdala, the centromedial group, to drive hypothalamic function (Walker et al., 2003). It also sends fibres to the basal forebrain and brainstem regions (Baydin et al., 2017), areas involved in pleasure and monoamine neurotransmitter regulation respectively.

The bed nucleus of the stria terminalis (BNST) is located at the caudal extremity of the stria terminalis and consists of a cluster of twelve to eighteen nuclei surrounding the caudal part of the anterior commissure, deep in the cerebral hemispheres (Dumont, 2009) (see also Chapter 1.4.1.). The BNST is a small but complex structure that extends from the nucleus accumbens region to the amygdala. As a processing centre in its own right, it receives inputs from the amygdala, which are relayed to the hypothalamus and brainstem nuclei (Dumont, 2009, Weller and Smith, 1982). The BNST is considered part of an extended amygdala complex and acts as a relay centre integrating autonomic, neuroendocrine and behavioural aspects of brain function (Lebow and Chen, 2016).

Imaging the structure of the stria terminalis is difficult due to its relatively small diameter, convoluted loop-like shape and close proximity to the fornix (Figure 1.11.). It is almost impossible to distinguish the stria terminalis from the fornix in standard clinical T1 or T2 images (Oishi et al., 2010). Three studies have used diffusion imaging in an attempt to visualise this white matter tract. One study demonstrated a pre and post commissural connectivity similar to the fornix (Kamali et al., 2015b), while another using the BNST as a seed region showed diffuse (but non-specific) connectivity of the nuclei across the hypothalamic and frontal areas (Kruger et al.,

2015). Both these techniques used probabilistic tractography. While useful for exploratory studies, this technique is not anatomically driven and thus not suitable for precise stereotactic localisation or regional quantification of diffusion metrics.

1.6.1. THE STRIA TERMINALIS IN DEPRESSION

Due to the inherent difficulties in imaging the stria terminalis, there has only been one study showing in vivo differences in humans in depression. Differences in stria terminalis integrity, but not fornix integrity was associated with differences in treatment outcomes in patients (Korgaonkar et al., 2014). In combination with the cingulum, decreased fractional anisotropy of the stria terminalis predicted remission with 62% accuracy. This however, was not an anatomically driven diffusion study and relied on probabilistic diffusion methods (see Chapter 2.3 for further details) and as such, the changes were only localised in areas presumed to be the stria terminalis. In animal studies, the stria terminalis, and more specifically the grey matter BNST, have been implicated in the stress response (LeDoux et al., 1988, Greenwood et al., 2005, Muigg et al., 2007, Luyck et al., 2017) and in the pathophysiology of depression (Stone et al., 2006, Crestani et al., 2010). Of note, stria terminalis activation resulting in induced stress can be attenuated by anti-depressant treatment (Muigg et al., 2007).

Deep brain stimulation of the stria terminalis, specifically the BNST, has been shown to improve depression symptoms in three studies of patients with MDD (Blomstedt et al., 2017, Neumann et al., 2014, Fitzgerald et al., 2018). Measurement of local field potentials in MDD patients during deep brain stimulation has suggested abnormalities around the area of the stria terminalis (Neumann et al., 2014). One study found that 40% of treatment-resistant MDD patients had sustained remission periods following DBS and that another 40% showed therapeutic improvements (Fitzgerald et al., 2018).

The evidence for the involvement of the stria terminalis in MDD and its potential as a therapeutic target for deep brain stimulation treatment of the disease suggests the need for a more precise neuroimaging technique to accurately localise, render and also quantify the structure in-vivo. A reliable and valid anatomically driven diffusion-weighted protocol for visualising the stria terminalis could allow investigation of the white matter tract as a potential biomarker for depression, or treatment response. In addition, accurate preoperative stereotactic localisation of the tract would be of benefit to surgeons in finding the tract prior to insertion of electrodes under MR guidance.

1.7. REDEEM STUDY

This thesis is derived from my work with the REDEEM (Research in Depression: Endocrinology, Epigenetics and neuroImaging) group at Trinity College Dublin over the past three years. This is a Health Research Board funded study (Grant No. 201651.12553, to Professor Veronica O'Keane) running over five years using patients referred to a psychiatrist for MDD. The study aims to compare the neurobiology of patients with MDD with healthy controls. Specifically, this study investigates differences in the HPA axis (through cortisol awakening response analysis), tryptophan/kynurenine metabolites (a biochemical pathway involved in the production of serotonin, a neurotransmitter important in MDD), the epigenetics of the glucocorticoid receptor and structural, functional, diffusion and spectroscopic neuroimaging in MDD patients. Eighty patients and eighty three controls have participated so far in the study, and their data is included in this thesis. The study is currently ongoing and data collection continues. Although I contributed to all aspects of data collection and analysis (i.e. endocrine, epigenetic and neuroimaging) during my time with the REDEEM group, this thesis is based mostly on the neuroimaging arm of the REDEEM study (specifically structural and diffusion imaging) (Chapters 3-6) with some analysis of cortisol awakening response data (Chapter 4).

CONTRIBUTION

In terms of data acquisition, I recruited, screened, scanned, obtained salivary and blood samples and administered clinical scales on approximately half the patients with MDD and almost two thirds of the controls. The rest of the patient data was obtained by other PhD candidates of the REDEEM group (Dr Kelly Doolin and Dr Chloe Farrell) prior to me joining the laboratory, following which I took over the recruitment and scanning of participants. All imaging data was processed and analysed by me, under the supervision of Dr Erik O'Hanlon (senior post-doctoral researcher – REDEEM group). Cortisol analysis (liquid chromatography-mass spectrometry) was undertaken by Dr Chloe Farrell of the REDEEM group in collaboration with Professor Brian Keevil at the University Hospital of South Manchester. All statistical analysis presented in this thesis was undertaken by me under the guidance of Dr Erik O'Hanlon. Professor Veronica O'Keane provided overall direction and guidance throughout, especially on analysis and interpretation of the data.

1.8 AIMS AND STRUCTURE OF THESIS

The aims in this doctoral thesis were to investigate the hippocampus, amygdala and their principle output tracts, the fornix and stria terminalis in depression, by comparing cases to controls using state of the art hippocampal and amygdala segmentation and diffusion imaging techniques. This investigation was aided by novel reassembly of *FreeSurfer* hippocampal and amygdala output into anatomical relevant composites as well as an original method for separating the fornix and the stria in diffusion imaging. Figure 1.11. shows these four structures and their relationship to each other.

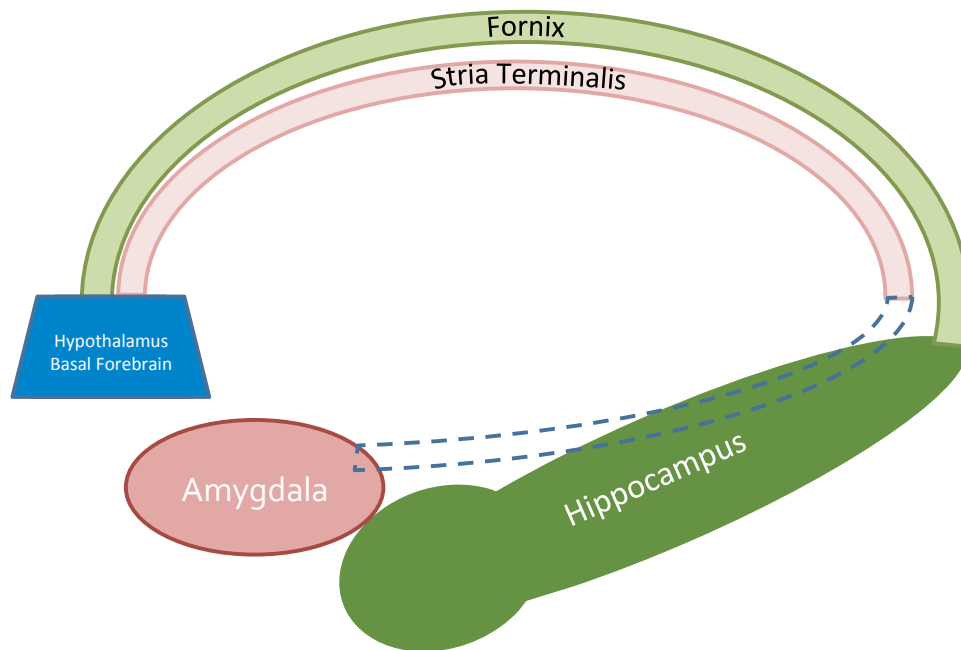


Figure 1.12. The hippocampus, amygdala, fornix and stria terminalis. Schematic diagram showing the relationship of all four structures examined in this thesis.

AIMS

This thesis has two broadly defined aims: to explore if

1. differences exist between depressed patients and controls for each of the four structures when these structures are measured as whole/complete structures,
2. differences exist between patients and controls for each of the four structures when these structures are examined at a more localised/substructural level.

The specific hypotheses for each limbic structure will be stated and explored within each relevant chapter 3-6. The acceptance or rejection of each null hypotheses will be addressed in Chapter 7.

STRUCTURE OF THESIS

The participant recruitment, clinical measures, neuroimaging, cortisol analysis and statistical techniques used to examine these structures are detailed in **chapter 2**.

The results concerning each of the four structures; i.e. hippocampus, amygdala, fornix and stria terminalis are presented and discussed fully separately in **chapters 3 to 6** respectively.

Finally, **chapter 7** provides a deeper discussion of the results as a whole, synthesising the results with reference to the current literature and potential future directions.

CHAPTER 2

METHODS:

SUBJECTS, SCANS AND STATISTICS

"The man who grasps principles can successfully select his own methods,
The man who tries methods, ignoring principles, is sure to have trouble"

Ralph Waldo Emerson
(attributed)

2.1 METHODOLOGY

This study used a case control design (Figure 2.1). Such a study design is an observational study where the investigator measures the exposures and outcomes in participants and reports the associations between them (Porta, 2008, Marshall, 2004). In this study the exposure was Major Depressive Disorder (MDD), and the outcomes were the potential differences in the structure and substructural volumes of the hippocampus and amygdala and the diffusion characteristics of the fornix and stria terminalis. Controls were individuals who had not been exposed to MDD, but otherwise were comparable to the cases. Case control studies have many advantages (relatively inexpensive and quick) and are particularly useful for generating useful hypotheses about potential associations. These hypotheses can then be used to inform more mechanistic prospective research questions (e.g. does the hippocampal change occur before or after depression starts).

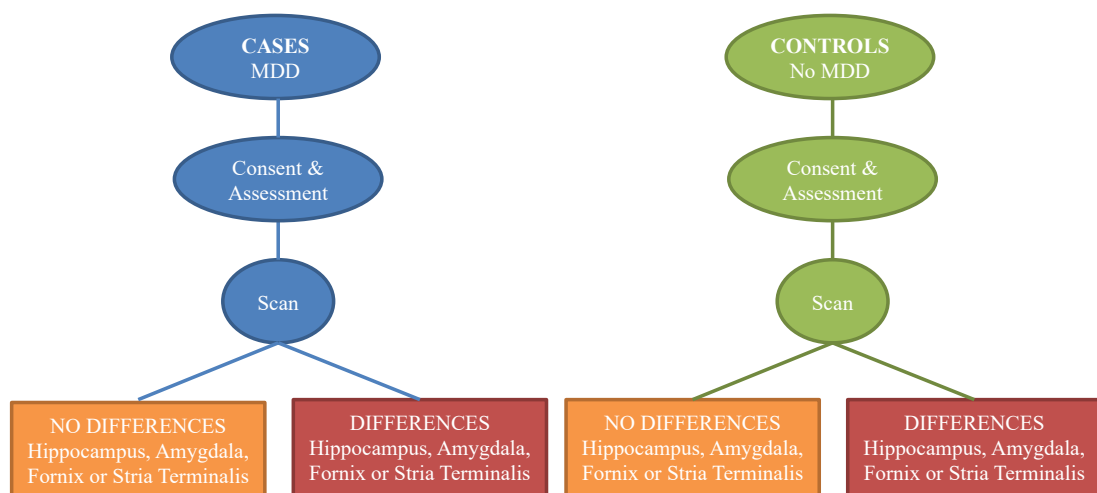


Figure 2.1 Case control design. A schematic diagram of the case control nature of this study. Outcomes were considered differences or no differences in each of the four substructures examined.

However, case control studies do have some disadvantages. The main limitations include recall bias (for example patients may not be able to recall all previous episodes of depression, or medications accurately), selection bias and an inability to establish causality from any of the potential differences between groups or associations with the independent variable (Schlesselman, 1982). This weakness is particularly important in the present study. Also, no temporal relationship can be inferred from our present design, due to the cross-sectional nature of the data collection – i.e. we are studying current neuroimaging findings in currently depressed individuals. It is not possible conclusively, using this sort of design, to separate out any hippocampal or amygdala differences found in the depressed group that are due to state or trait depression. Other possibilities also ought to be considered when using this type of design; e.g., instead of MDD causing these changes, these changes may have occurred as a consequence MDD, or that even another mediating factor could exist to explain the associations (i.e. the neuroimaging findings and depression are not directly linked causatively).

In this chapter I will now outline my methods in detail in four sections. First, I will discuss patient and control recruitment and the assessment of depression these individuals. This will be followed by a detailed account of the structural and diffusion neuroimaging methods used, including two novel techniques used for the analysis of structural and diffusion data. Thirdly, I will briefly discuss cortisol collection and analysis which has relevance for the amygdala part of the study. Finally, I will review the statistical analysis used for each of the structures examined in the thesis. Each following chapter will also give a brief summary of the particular methods used for that chapter.

2.2 PARTICIPANTS

Depressed patients were recruited from the psychiatric outpatient clinic at Sheaf House in Tallaght Hospital, Tallaght, Dublin 24, which is the headquarters for the Tallaght area Community Mental Health team. Study participants were also recruited from the Mary Mercer Health Centre, Jobstown, Dublin 24, which is a primary care clinic for the outpatient department of psychiatric services in Tallaght. The Health Services Executive (HSE), the national public health service for Ireland, administrates both centres. Patients were initially referred either by their GP or through the Emergency Department. All patients who presented to these clinics with depression were considered for the study. All patients considered were offered the choice to participate in the study, by their treating doctor. Patients were given a patient information sheet at this time and could refuse entry into the study. In total eighty-three patients were recruited.

Patients were initially screened by a consultant psychiatrist, VO'K, the supervisor of this thesis, at clinical interview. Exclusion criteria for patient entry into the study included contraindications to MRI, history of illicit substance abuse, head trauma, and any significant medical conditions. Patients with a history of major mental illness, apart from depression and those with a personality disorder were also excluded. In total eighty patients were recruited.

Controls were recruited through an active database of willing participants, as well as family/friends of members of the REDEEM team. The same exclusion criteria applied as the patient cohort, but with the added exclusion criteria that they never had a diagnosis of depression. In total eighty-three patients were recruited.

All participants underwent a MINI (Mini International Neuropsychiatric Interview) structured clinical interview for DSM-IV (American Psychiatric Association, 1994) and

the Hamilton Depression Rating Scale (Hamilton, 1960) (HAM-D) at TCIN (Trinity College Institute of Neuroscience) prior to scanning.

The MINI interview is a brief, structured interview developed for the major Axis I psychiatric disorders including MDD (Hergueta et al., 1998, Sheehan et al., 1998). For this research study, modules A-C were completed for each participant. These modules cover on (A) Major Depressive Episode and Major Depressive Episode with Melancholic Features, (B) Dysthymia, and (C) Suicidality. For participation in this study, every patient was required to meet diagnostic criteria for a current Major Depressive Episode according to DSM-IV. Note there has been no significant change in the basic criteria required for the diagnosis of depression between DSM-IV and DSM-5 (Tyrer, 2014).

The Hamilton Depression Rating Scale (HAM-D) is a validated and standardised as a measure of depression (Olden et al., 2009). It requires the core experiences of MDD such as low mood and anhedonia for at least two weeks and includes other questions that address the range of symptoms and experiences of depression including suicidal ideation, insomnia, anxiety, somatic symptoms, sexual function, gastrointestinal symptoms and more (Williams, 2001). Even though often criticized for inflexibility in the diagnosis of MDD in clinical settings (for example it does not specifically measure atypical or agitation depressed states), the HAM-D is considered the 'gold standard' for depression research (Bagby et al., 2004). Both the MINI structured clinical interview for DSM-IV and the HAM-D are included in Appendix 3.1 and 3.2 respectively.

Entry criteria for patients were that they must have a current DSM-IV diagnosis of Major Depressive Disorder with a HAM-D of 17 and above. A HAM-D score greater than 17 is considered moderately depressed, and a score greater than 22 is considered severely depressed (Williams, 2001). Entry criteria for controls consisted of an absence of both an active or previous MINI diagnosis (including MDD) and a HAM-D

less than 8 (considered not depressed). A consensus meeting between the consultant psychiatrist (VO'K) and research team at TCIN was held prior to every subject entry into the study to determine if the patient met the clinical criteria for inclusion and were not contraindicated to have an MRI. Depressed patients were also subdivided into First Presentation Depression (FPD) and Recurrent Depression (RD) groups, based on previous attendance to psychiatric services. FPD patients had no previous attendance at psychiatric services but could have been treated by their GP for depression. RD patients were those who had previously attended any psychiatric services more than once for at least one episode of diagnosed MDD.

Fully written informed consent using a detailed information sheet was obtained at the clinic during recruitment and also prior to scanning at TCIN (see Appendix 2.1). Other clinical data such as how many months in total with MDD over the lifespan and medication history were obtained. Patients were given cortisol Salivette® tubes in clinic and ask to complete them at home before the scan. The research team at TCIN rechecked inclusion and exclusion criteria prior to every subject commencing scanning, with particular focus on participant safety and MRI contraindications (see Appendix 2.4). MRI scanners have large electromagnets and also emit intense radiofrequency pulses. Metal objects in a participant's body could be at risk of being pulled out due to the strength of the electromagnetic or be at risk from thermal heating due to the radiofrequency pulses. Most metal objects are thus contraindicated in MRI. Some non-ferrous metallic objects such as tooth fillings may also affect the image acquisition quality in areas close to the MRI field of view. This was particularly relevant for this study, investigating the temporal lobe regions, found just above the maxilla. Tooth fillings may interfere with the signal from this brain region resulting in signal distortion or fall-out. The complete list of contraindications and patient checklist is found in Appendix 2.4. Ethical approval was obtained from the Tallaght Hospital and St James Hospital Joint Research Ethics Committee. This committee operates on the principles of the Declaration of Helsinki (WMA, 2013).

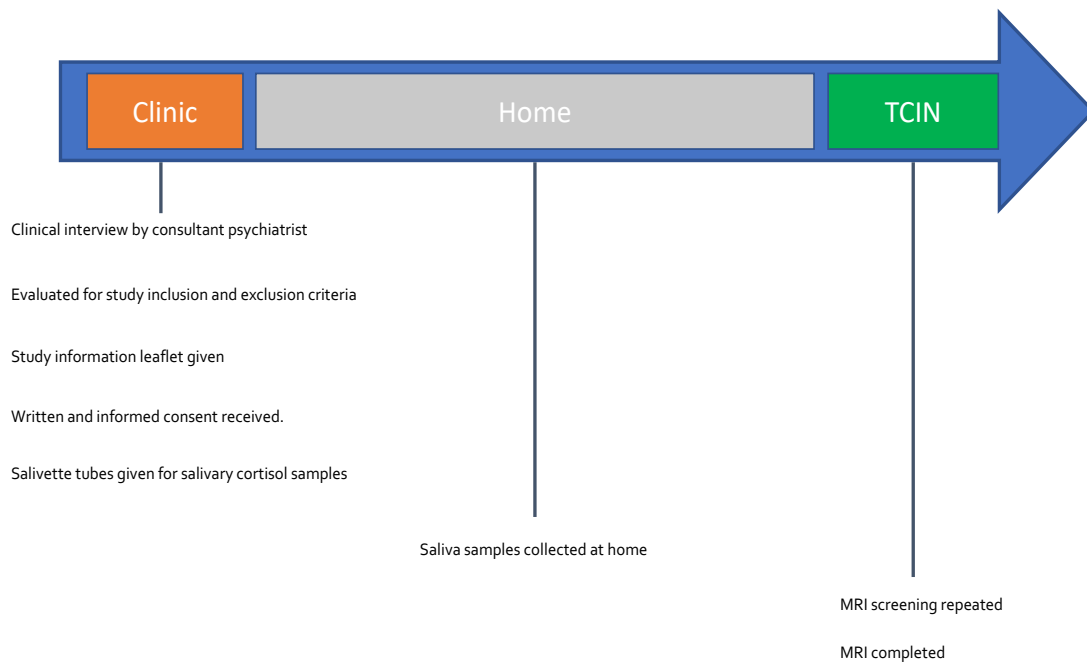


Figure 2.2. Participant timeline. Total time for the participants from clinic to scan was 1 week. The saliva samples could be taken any morning before the scan, but preferably a 'normal' day (see Chapter 2.8.). TCIN, Trinity College Institute of Neuroscience.

2.3 MAGNETIC RESONANCE IMAGING

All patients underwent Magnetic Resonance Imaging (MRI). MRI uses the magnetic properties of hydrogen nuclei (protons) in strong magnetic fields with radiofrequency energy to generate detailed images (Berger, 2002). The radiofrequency waves cause the protons to move to a high-energy state. After the radiofrequency pulse is switched off, the time is measured for the protons to relax from their high-energy state. The time taken for the magnetic vector to return to its resting state is known as T_1 and the time needed for the axial spin to return to its resting state is T_2 . With T_1 images, grey matter (cortex) is grey and white matter (axons) is light in colour, whereas T_2 images reverse this grey/white matter dichotomy. T_2 -FLAIR (Fluid Attenuated Inversion Recovery) is essentially a very long T_2 relaxation that allows for complete suppression of the water signal (i.e. the CSF in the brain), generating clearer images (Westbrook and Talbot, 2018). See Figure 2.3. and Table 2.1.

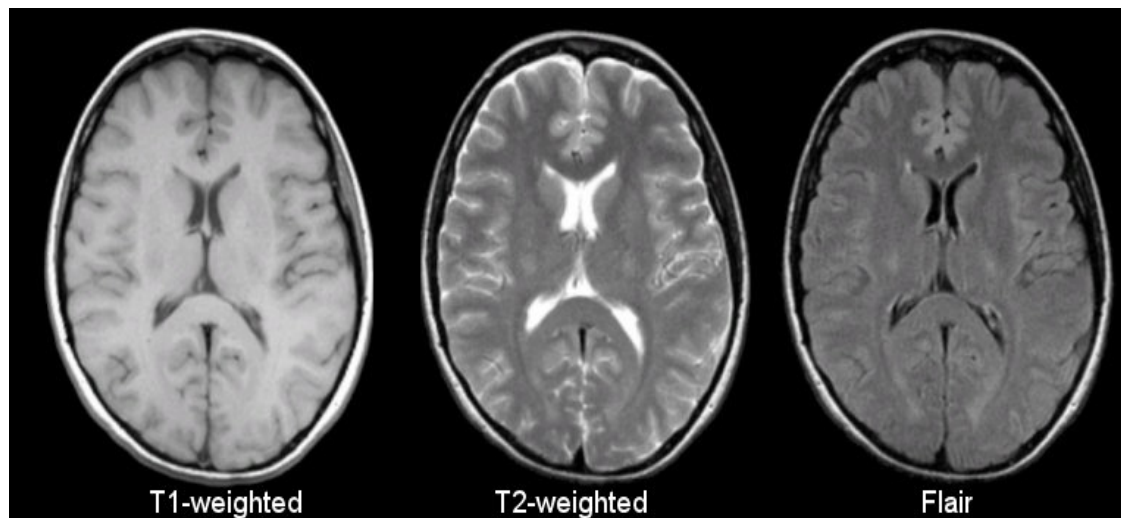


Figure 2.3. T₁, T₂ and T₂-FLAIR images. See Table 2.2 for comparisons of tissue properties of each images.

Tissue	T1	T2	T2-FLAIR
CSF	Dark	Bright	Dark
White matter	Light	Dark Gray	Dark Gray
Grey matter	Gray	Light Gray	Light Gray

Table 2.1. T₁, T₂ and T₂-FLAIR properties. The table corresponds to grey and white matter and CSF in Figure 2.2 above. CSF, cerebrospinal fluid.

DIFFUSION WEIGHTED IMAGING

Diffusion weighted imaging (DWI) is an MRI technique that aids the study of white matter pathways or tracts. Essentially DWI involves the measurement of the random Brownian motion of water molecules (Jones et al., 1999) and exploits the fact that water diffuses characteristically within white matter tracts, i.e. water diffuses primarily along the axon and parallel to the longitudinal axis, with very limited perpendicular movement (Yamada et al., 2009, Koch et al., 2014) (See Figure 2.4.). Diffusion predominantly in one direction (e.g. along a fibre tract, but not perpendicular to it) is known as anisotropic diffusion. The orientation and integrity of white matter tracts in the brain can be determined by measurements of anisotropy and microstructural indices (i.e. diffusion metrics) (Murphy and Frodl, 2011, Mori and Aggarwal, 2014) (see Chapter 2.7.3. for equations). Diffusion metrics are used in DWI to characterise diffusivity and consequently infer white matter tract properties (Table

2.2.). Thus, DWI allows for the non-invasive *in-vivo* virtual dissection, three-dimensional visualisation and quantification of the architecture of white matter fibres in the brain (Catani and Thiebaut de Schotten, 2008).

	Fractional Anisotropy	Mean Diffusivity	Axial Diffusivity	Radial Diffusivity
Definition	Microstructural integrity	Inverse measure of membrane density	Variable in WM changes in pathology	Increases in WM with demyelination
WM Characteristics				
Dense Axonal Packing	High	Low	Unaffected	Low
High myelination	High	Low	High	Low
Axonal degeneration	Low	High	Low	High
Demyelination	Low	High	Unaffected	High

Table 2.2. DWI microstructural indices. A summary of diffusion metrics. See Chapter 2.7.3. for calculation of individual metrics. Their relationship under different conditions is also shown - adapted from Feldman et al. 2010. DWI, diffusion weighted imaging; WM, white matter.

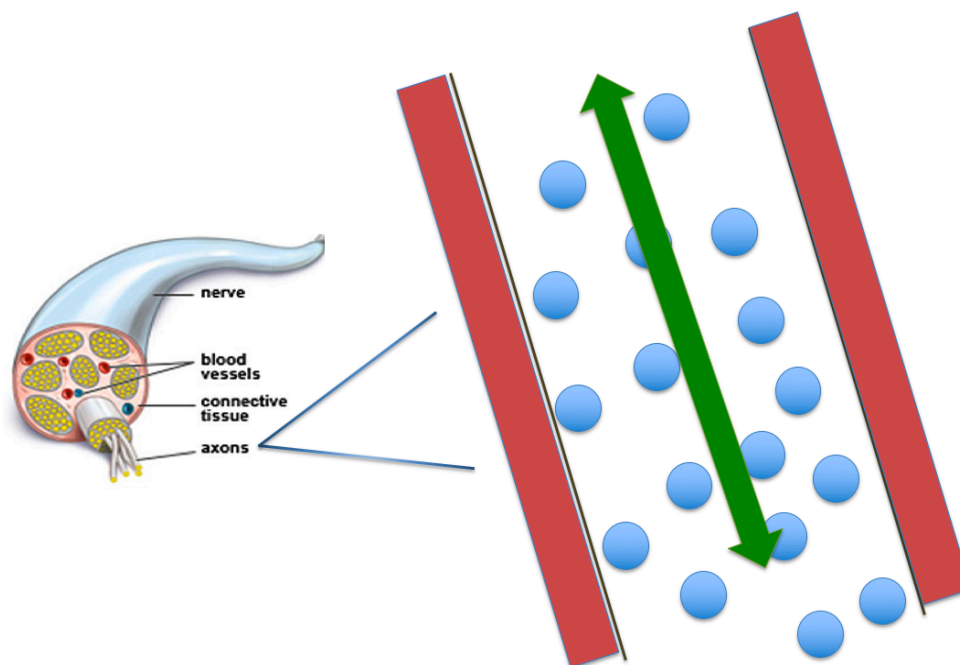


Figure 2.4. Principles of diffusion along an axon. Water molecules (shown in blue) can only diffuse parallel to the axon (along the green arrows). Water cannot diffuse perpendicular to the axon due to the physical boundaries (red lines). Through this we can infer the direction of white matter tracts.

Diffusion tractography is a method of extracting trajectories of specific white matter pathways from DWI data (Pujol et al., 2015). Tractography involves the use of two main types of algorithms, probabilistic and deterministic (Basser et al., 2000). Probabilistic tractography involves calculating the probability of multiple diffusion directions within each voxel (a three-dimensional pixel of tissue) (Jones, 2008). Deterministic tractography involves assuming that there is one main diffusion direction within each voxel (Yamada et al., 2009, Jones, 2008). Deterministic tractography involves the use of at least two regions of interest (ROIs) to map neuronal connections (Jones, 2008). ROIs isolate specific tracts from whole brain tracts data using Boolean logic. ROIs are chosen based on prior neuroanatomical knowledge and allow for the determination of the structural characteristics of WM bundles in the area of interest (Murphy and Frodl, 2011). As distinct tracts with known neuroanatomical characteristics and fixed start and end points, both the fornix and stria terminalis make good candidates for deterministic tractography. As such, this study uses deterministic tractography.

One major limitation of DWI is that voxels contain more than a single type of tissue and areas of crossing fibres can be difficult to depict. Tractography can consequently be adversely impacted by the presence of other white matter structures that lie in close proximity to the tract under investigation. These crossing fibres can lead to changes in the angle and volume fractions of crossing white matter fibres resulting in partial volume effects (Dell'Acqua et al., 2013). This can lead to inaccurate or misleading diffusion metrics (Yamada et al., 2009). Constrained spherical deconvolution (CSD) is a potential solution to the limitations of the multiple fibre populations or intravoxel fibre heterogeneity. CSD assumes constant anisotropy along fibres and estimates the distribution of all possible fibre orientations within each voxel (Emsell et al., 2017, Dell'Acqua et al., 2013). This method introduces a function known as the fibre orientation distribution (FOD). The FOD is defined as the intravoxel distribution of fibre orientations and gives information about the proportion of orientations as well as the distinct weight and orientation of each

potential fibre (Dell'Acqua et al., 2013, Tournier et al., 2004). This allows for more convoluted tracts such as those of the limbic system to be accurately explored.

2.4 MRI ACQUISITION

All data were acquired on a Philips Intera Achieva 3.0 Tesla MR system (Best, Netherlands) equipped with a 32-channel head coil in TCIN. Participants underwent four modalities of MRI in the following order; structural (T1, T2-FLAIR), magnetic resonance spectroscopy, diffusion-weighted imaging, and resting-state functional MRI. Total scanning time was approximately an hour. Only structural and diffusion imaging form the basis of this doctoral thesis. The spectroscopy and functional data will form part of my post-doctoral work.

2.4.1. T1 IMAGING

180 axial high-resolution T1-weighted anatomical images were acquired (T1W-IR 1150 sequence, TE = 3.8 ms, TR = 8.4 ms, FOV 230 mm, 0.898 x 0.898 mm² in-plane resolution, slice thickness 0.9 mm, flip angle alpha = 80). These parameters yielded an image with an isotropic resolution of 0.9 mm³. This scan lasted 6 minutes.

2.4.2. T2-FLAIR IMAGING

60 axial T2-FLAIR images (TE = 120 ms, TR = 2800 ms, FOV 230 mm 0.49 mm x 0.49 mm in-plane resolution, slice thickness 3 mm, flip angle alpha = 80). These parameters yielded an image of 0.49 mm x 0.49 mm x 3.0 mm with the larger slice thickness in the axial plane and the smaller the smaller in-plane resolution in the coronal and sagittal planes. This approach ensured that the highest resolution was along the anteroposterior hippocampal axis, allowing a more detailed survey of the hippocampal substructures. This scan lasted 4.5 minutes.

2.4.3. DIFFUSION-WEIGHTED IMAGING

Whole brain, High Angular Resolution Diffusion Imaging (HARDI) (Jones and Leemans, 2011, Jones et al., 2013b) was acquired using a spin-echo echo-planar imaging (SE-EPI) pulse sequence (TE = 52 ms, TR = 11,260 ms, flip angle $\alpha = 90^\circ$), FOV 224 mm, 60 axial slices, 2mm³ isotropic voxels, b-value = 1500 s mm⁻² in 61 non-collinear gradient directions. This scan lasted 16 minutes.

2.5 HIPPOCAMPAL & AMYGDALA SUBSTRUCTURE ANALYSIS

All structural images (T1 and T2-FLAIR) were inspected manually to ensure there were no issues with corrupted data, subject movement artefacts or poor/failed acquisition. Cortical reconstruction and volumetric segmentation was performed with the *FreeSurfer 6.0* image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>) (Fischl, 2012b, Fischl and Dale, 2000). *FreeSurfer* is a free brain imaging software package developed by the Athinoula A. Martinos Center for Biomedical Imaging at Massachusetts General Hospital, Massachusetts, United States. It designed specifically for analysing research MRI scan data (Fischl, 2012a). It contains tools to conduct both volume-based and surface-based analysis and can automatically label both cortical and subcortical structures according to predefined maps.

FreeSurfer 6.0 contains a newly updated hippocampal and amygdala analysis module (<http://FreeSurfer.net/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala>) that was used for this analysis. This module was developed primarily by Juan Eugenio Iglesias of University College London in association with Massachusetts General Hospital (Iglesias et al., 2015, Saygin et al., 2017). The hippocampal-amygdala tool uses a probabilistic atlas build upon very high-resolution ex-vivo T1 MRI data (0.1-0.15 mm resolution; 0.13 mm average isotropic resolution) as well as more conventional (1 mm isotropic) in-vivo MRI datasets (Iglesias et al., 2015). The model uses a Bayesian inference algorithm (Van Leemput, 2009) to calculate the topology and reveal

substructures depending on the distinct contrast differences of adjacent substructures.

The hippocampal-amygdala module is an evolution of the previous hippocampal-only module released initially with *FreeSurfer 6.0*. This in turn, is an improvement of the more rudimentary hippocampal solo module found in *FreeSurfer 5.3*, which as well as only calculating seven substructures, demonstrated well documented problems with specific subfield volume errors (Wisse et al., 2014). The improved, most up to date version can discriminate up to 13 hippocampal subfields (Table 2.2) as well as segmenting these structures proportionally along the hippocampal anteroposterior axis. Nine amygdalar nuclei can also be discerned (Table 2.3). Joint segmentation of the amygdala and hippocampus simultaneously guarantees that these structures do not overlap or leave gaps, ensuring a more consistent segmentation.

FreeSurfer requires MATLAB for operating the hippocampal-amygdala module. MATLAB (Matrix Laboratory) (www.mathworks.com) is a proprietary programming language and multi-paradigm numerical computing environment developed by Mathworks Inc. and allows matrix manipulations, plotting of functions and data and implementation of algorithms (Sobie, 2011). Due to the large number of images (326 images in total including both T1 and T1) and the extremely large number of substructures (13 hippocampal plus 9 amygdalar substructures on each side across 163 subjects = 7172 individual substructures), the data was processed through the high-performance computers at Trinity College Dublin. I wish to expressly thank Dr. Erik O'Hanlon for his computing expertise with this aspect of my research.

FreeSurfer 6.0 can also generate an estimate of total intracranial volume (eTIV) using the T1 images. The technical details of the procedures involved in running *FreeSurfer*, generating eTIV and implementing the hippocampal toolbox are described elsewhere (Fischl et al., 2002b, Dale et al., 1999, Desikan et al., 2006, Fischl et al., 1999, Fischl et al., 2004, Reuter et al., 2012). *FreeSurfer* optimization parameters were

employed to generate more accurate outputs. These included activation of the “3T” data flag, and the use of both T1 and T2 data to improve internal hippocampal subfield segmentation and boundary identification. T2 imaging is particularly useful for hippocampal segmentation due to the ability to highlight white matter within the hippocampus (Mueller et al., 2007, Kerchner et al., 2012, La Joie et al., 2013, Wisse et al., 2015, Wisse et al., 2014), and by combining both T1 and T2 data it was hoped to increase the sensitivity of the hippocampal analysis. Such increased sensitivity by combining modalities was shown in an Alzheimer’s disease cohort by combining T1 and T2 imaging (Iglesias et al., 2015). *FreeSurfer* outputs included both visual representations of the individual substructures as well as volumes in mm³ of each region. T1 only hippocampal data was also calculated and is presented in Appendix 4 for comparison. The amygdala component of the module requires only T1 images. To date there is no published study which has used new *FreeSurfer* amygdalar module in any illness - apart from the original validation study (Saygin et al., 2017).

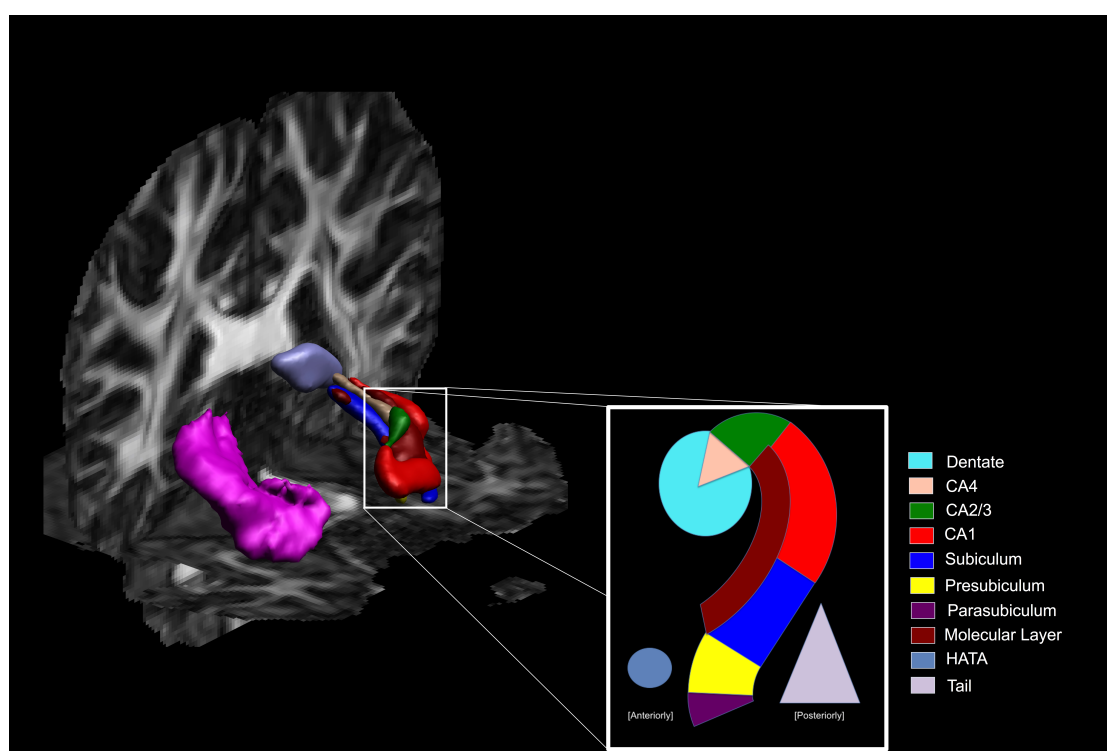


Figure 2.5. *FreeSurfer* 6.0 hippocampal output. The right hippocampus is in solid magenta. The left hippocampus shows segmented *FreeSurfer* substructures (not all are visible). A schematic cross-section of the left hippocampus is shown. This schematic diagram shows the relative sizes of substructures. CA, Cornu Ammonis; DG, Dentate gyrus; ML, Molecular Layer; Subic, Subiculum.

Substructure	Definition	Comments
CA1	Lies between CA2/3 and subiculum. The molecular layer separates it from the fissure.	Key component of the trisynaptic circuit, having connections with the CA3 and subicular output regions.
CA2/3	Combined CA2 and CA3 subfields as <i>FreeSurfer</i> is unable to distinguish between them. Lies between the hilar area of the dentate (CA4) and CA1.	Key component of the trisynaptic circuit and origin of Schaffer collaterals. Site of synapses of mossy fiber pathway. Important in encoding short term memory.
CA4	Lies within the hilum of the dentate. Contains the polymorphic layer of the dentate gyrus.	Functionally considered part of the dentate.
Dentate	Contains only the granule cell and molecular layer of the dentate. Begins about one-third of the way through the hippocampal head.	Input region of trisynaptic hippocampal circuit. Important for pattern separation and site of neurogenesis. Origin of mossy fiber pathway.
Subiculum	Lies between the CA1 and presubiculum. The molecular layer separates it from the fissure.	Key output substructure of hippocampal circuitry.
Presubiculum	Brodman Area 27. It lies between subiculum and the parasubiculum along the fissure.	Contains an abundance of grid, border and head direction cells. Important for scene recognition and visospatial processing.
Parasubiculum	Brodman Area 49. Lies between entorhinal cortex and presubiculum, along the base of the fissure.	Contains an abundance of grid, border and head direction cells. Important for scene recognition and visospatial processing.
Tail	The posterior end of the hippocampus. Defined by the first coronal slice showing contact with the fornix and all regions posterior to this.	Contains all principal substructures similar to the anterior hippocampus but in varying proportions.
Molecular layer	Upper layer of the CA1-3 and subiculum. Lies adjacent to the fissure.	Relatively neuronal cell body free. Contains inflammatory cells, connective tissue and axons of connecting neurons.
HATA	Hippocampal Amygdala Transition Area. Essentially lies between the hippocampus and amygdala dorsorostrally.	Postulated to be a component of the amygdalo-hippocampal network.
Fimbriae	White matter fiber bundle on temporal aspect of hippocampus.	Coalesces to form the fornix, the main output tract of the hippocampus.
Fissure	CSF space between the molecular layer of the hippocampal gyrus and the dentate gyrus.	

Table 2.3. *FreeSurfer* computed hippocampal substructures. All eleven computed substructures, describing definitions and functions if appropriate. CA, cornu ammonis; CSF, cerebrospinal fluid; HATA, hippocampal amygdala transition area.

Table 2.4. (on next page) *FreeSurfer* computed amygdala substructures. All nine computed substructures, describing definitions and functions if appropriate.

Structure	Definition	Comments
Lateral	The largest single nucleus of the amygdala, and probably the easiest to discriminate. Classic wedge/triangular shape. Dorsolateral to the basal nucleus along the anteroposterior extent of the amygdala.	5 subnuclei; dorsal anterior, intermediate, ventral, dorsomedial and dorsolateral. Main receptor of sensory inputs reaching the amygdala. Rich in vasoactive intestinal peptide. Striated appearance due to fibers of uncinate fasciculus.
Basal	Borders the lateral nucleus ventromedially.	The main target of afferents from the prefrontal cortex and dorsomedial thalamus. 3 subdivisions; magnocellular, parvocellular and intermediate.
Accessory Basal	This lies ventral to the basal nucleus and adjacent to the hippocampus caudally.	Has 3 subdivisions similar to basal; magnocellular, parvocellular and intermediate. Local processing of sensory inputs and transmits to central and medial nuclei.
Paralaminar	Wedge inferior the basal, lateral and cortico-amygdaloid transition areas.	Relatively expanded in humans and non-human primates. Site of plasticity of the amygdala. Dense serotonergic connections.
Central	Dorsal to the accessory basal nucleus. Often circular or oval in shape.	Key output substructure of amygdala circuitry, primarily to the lateral hypothalamus via the amygdalofugal pathway. Four subdivisions. Considered part of the extended amygdala.
Medial	Visible along anteroposterior extent of amygdala, medially. Slim and elongated.	Four subdivisions. Considered part of the extended amygdala. Important output to the medial hypothalamus via the stria terminalis
Anterior Amygdala Area	The most anterior end of the amygdala.	Contains an abundance of grid, border and head direction cells. Important for scene recognition and visospatial processing.
Cortico-Amygdaloid Transition Area	The most medial border of the amygdala.	A confluence of the medial basal, paralaminar and periamygdaloid areas.
Cortical	Most superficial and smallest nucleus, bordering the accessory basal medially and the medial nucleus rostrally.	Covered by a thin cortical layer, the periamygdaloid area. Can be divided into anterior and posterior cortical nuclei. Related to and connected with olfactory areas.
<u>Composites</u>		
Whole amygdala	Lateral, basal, accessory basal, paralaminar, central, medial, cortical, anterior amygdala area, cortico-amygdaloid transition area.	Total combined nuclei of amygdala.
Laterobasal	Lateral, basal, accessory basal, paralaminar.	Largest nuclear group. Cortical-like neurons (either pyramidal with spiny dendrites (class I) or smaller neurons with nonspiny dendrites (class II)). Reciprocal connections with the cortex and thalamus. Input area.
Centromedial	Central, medial.	Smallest nuclear group. Striatal-like medium sized spiny neurons with connections to neuroendocrine and autonomic hypothalamus. Output area.
Superficial	Cortical, anterior amygdala area, cortico-amygdaloid transition area.	Olfactory-like region, with three cortical layers. Possibly concerned with social processing.

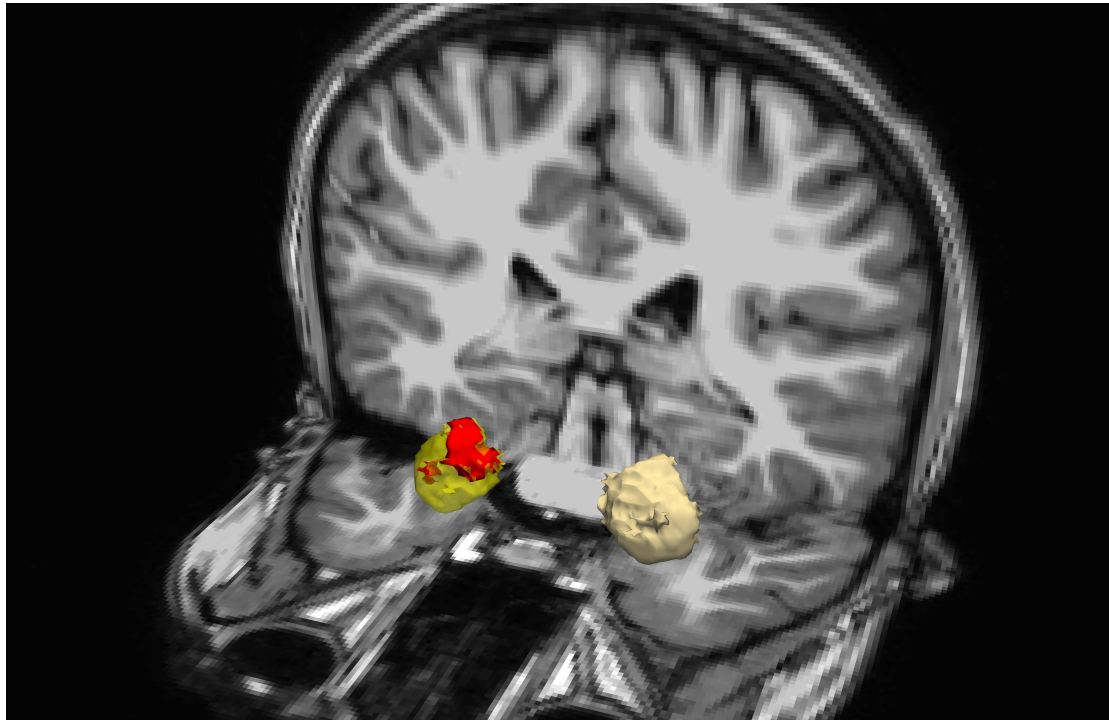


Figure 2.6. *FreeSurfer* 6.0 amygdala output. The left hippocampus is in solid cream. The right hippocampus in lime green shows the segmented *FreeSurfer* combined centromedial nuclei in red.

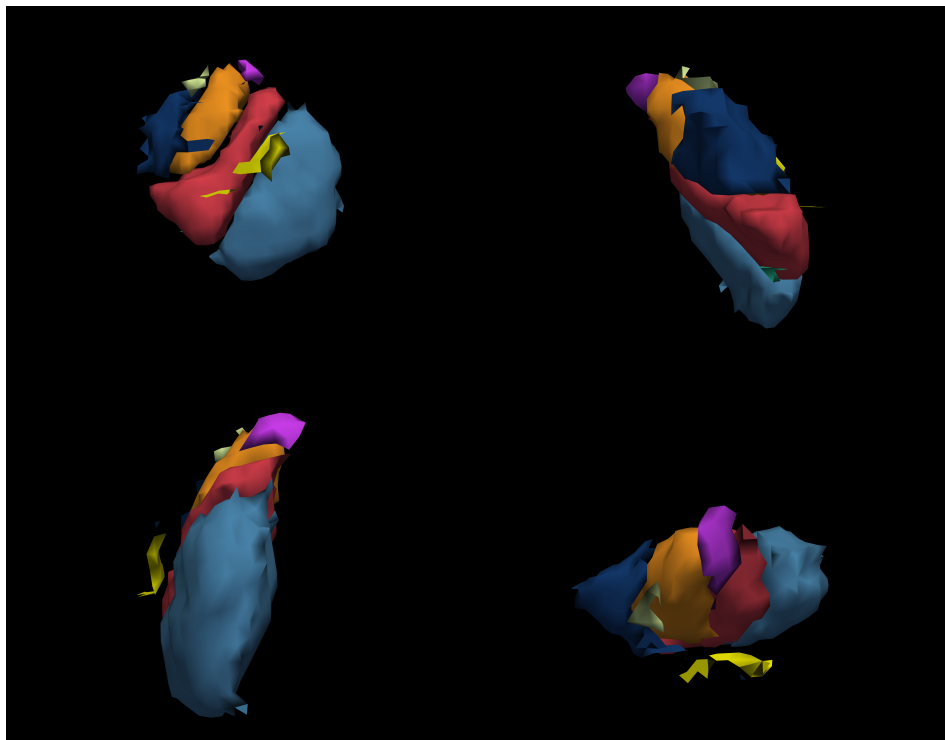


Figure 2.7. *FreeSurfer* 6.0 Amygdala substructures output. a) sagittal medial, b) coronal c) sagittal lateral and d) axial views of amygdala computed substructures. *FreeSurfer* 6.0 computes nine substructures or nuclei as shown here. Different colours represent specific nuclei; green, medial; dark blue, corticoamygdala transitional area; orange, accessory basal; red, basal; purple, central; off-white, cortical; yellow, anterior amygdaloid area; light blue, lateral; turquoise, paralaminar.

2.5.1. NOVEL COMPOSITE MEASURES

Individual hippocampal and amygdala substructures were combined together according to anatomical and functional criteria to form seven hippocampal and four amygdala composite measures. Although the individual substructures in Table 2.3. and Table 2.4. can provide some insights into the form and function of these regions in depression (Zimmerman et al., 2016), these substructures should not be viewed in isolation, but rather as collaborative interconnecting processing hubs across circuit groups which function both within and without the hippocampus (de Wael et al., 2018) and amygdala (Janak and Tye, 2015). Some of these substructures have overlapping hodological properties whereas others have similar anatomical properties. Therefore, based on known anatomical and connectivity properties of the substructures, I have devised novel composite measures for both the amygdala and hippocampus to further investigation of the anatomical and functional properties of the combined regions. These were calculated by simply summing the outputted volumes of the appropriate substructure volumes from *FreeSurfer* prior to statistical analysis.

HIPPOCAMPAL COMPOSITES

Varying definitions of whole hippocampal volume exist (Andersen, 2007, Schultz and Engelhardt, 2014). To resolve this, I developed three measures of total hippocampal volume depending on neuroanatomical definitions. The hippocampus proper (HP) consists of the classic allocortical cornu ammonis (CA1-4) regions only – i.e. the hippocampal core (Andersen, 2007); the hippocampal formation (HF) involves a more functional definition of the hippocampus and consists of the CA regions and includes the dentate region and subiculum (Schultz and Engelhardt, 2014). Finally, all the computed substructures (including the white matter structures but excluding the hippocampal fissure) were summed to together to create a fully inclusive, but non-specific hippocampus extended (HE). See Table 2.5.

As well as whole hippocampal volume definitions, four exploratory anatomico-functional definitions of hippocampal regions were created. These included the

Combined Dentate consisting of dentate and CA₄ (as CA₄ is considered functionally part of the dentate); *CA-Only* composite (exclusive CA regions only, not including the CA₄ region for the aforementioned reason); *Combined Dentate/CA* region corresponding the path of the trisynaptic circuit, and combining all the dentate and CA substructures, but excluding the subiculum and; *CA2-4* regions consisting of CA2-4 only. See Table 2.6

AMYGDALA COMPOSITES

The amygdala is formed from a collection of substructures (nuclei) that can be clustered into three broad groups according to the functional and anatomical literature; laterobasal, medial and superficial (Heimer et al., 1999). See Table 2.6. and Figure 2.6. and Figure 2.8.

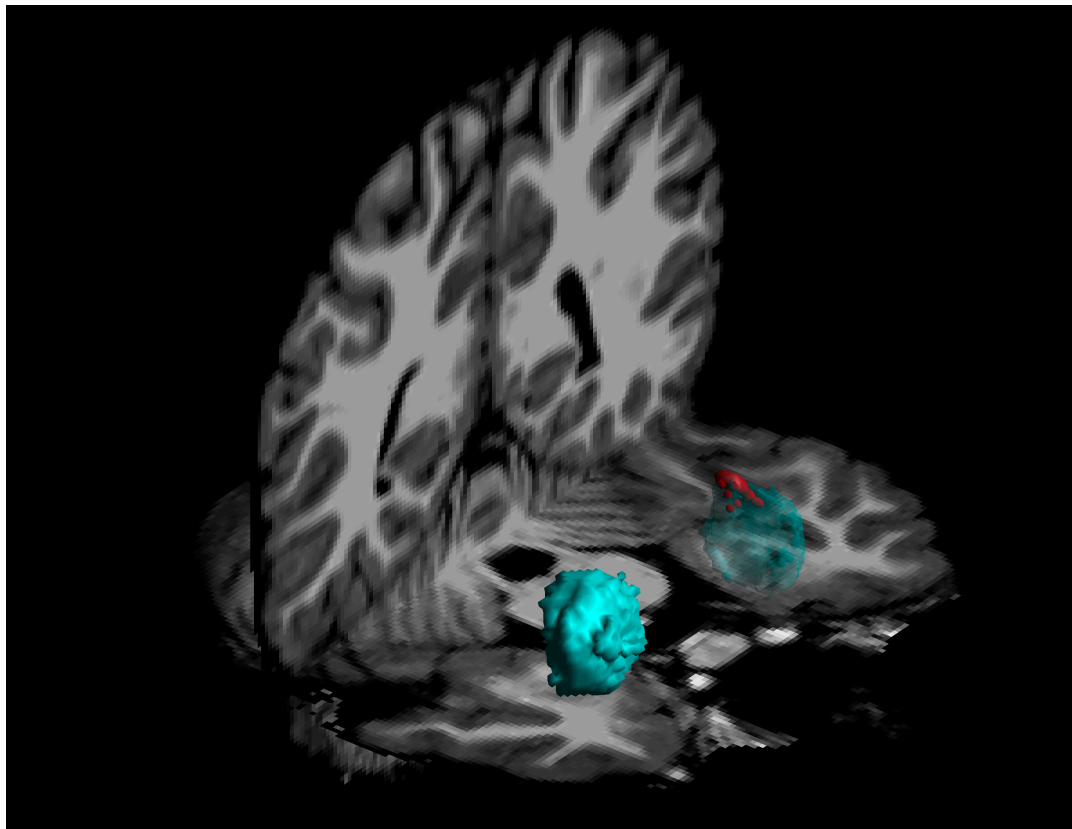


Figure 2.8. Amygdala and centromedial group. Oblique T1 axial and sagittal image showing a rendered right amygdala in turquoise. The left amygdala is rendered semi-transparent with centromedial nuclei seen in red.

Composite Measures	Substructure volumes added	Comment
Hippocampal Extended	CA1, CA2/3, CA4, Dentate, Subiculum, Tail, Molecular Layer, Presubiculum, Parasubiculum, HATA, Fimbriae.	All FreeSurfer hippocampal computed substructures except the fissure.
Hippocampal Formation	CA1, CA2/3, CA4, Dentate, Subiculum, Tail	All computed substructures that contain CA, dentate and subiculum components.
Hippocampal Proper	CA1, CA2/3, CA4	Classic anatomical hippocampus components only.
Combined Dentate	CA4, Dentate	Anatomical and functional dentate components.
CA Only	CA1, CA2/3	Exclusive CA components only, not including the molecular layer which contain subicular components.
Combined Dentate/CA	Dentate, CA1, CA2/3, CA4	Dentate and CA regions corresponding to the Trisynaptic circuit.
CA2-4	CA2/3, CA4	Exploratory composite to investigate significance of these regions in FPD.

Table 2.5. Total hippocampal and anatomical composites. Composites are created by summing component substructures. The component parts of three composites of total hippocampal volume and three anatomical composites are detailed. See Figures 2.4 for further information. Although the hippocampal fissure is also calculated by *FreeSurfer*, it is not summed into the Hippocampal Extended definition, as it not a true substructure, but the space between the hippocampal and dentate gyri. CA, Cornu Ammonis; HATA, Hippocampal Amygdalar Transition Area. See Figure 2.5.

Composite Measures	Substructure volumes added	Comment
Whole amygdala	Lateral, Basal, Accessory Basal, Paralaminae, Central, Medial, Cortical, Anterior Amygdala Area, Cortico-Amygdaloid Transition Area	Total combined nuclei of amygdala
Laterobasal	Lateral, Basal, Accessory Basal, Paralaminae	Largest nuclear group. Cortical-like with reciprocal connections with the cortex. Input area
Centromedial	Central, Medial	Smallest nuclear group. Striatal-like with connections to hypothalamus. Output area.
Superficial	Cortical, Anterior Amygdala Area, Cortico-Amygdaloid Transition Area	Olfactory-like region. Possibly concerned with social processing

Table 2.6. Total amygdala and anatomical composites. Composites are created by summing component substructures. The component of total amygdala volume and three anatomical composites are detailed. See Figure 2.6., Figure 2.7. and Figure 2.8.

2.6. DIFFUSION WEIGHTED IMAGING ANALYSIS

Analysis of the diffusion data consisted of three stages; 1) pre-processing of the raw diffusion data; 2) whole brain tractography and 3) manual 'dissection' of the fornix and stria terminalis from the whole brain tractographs. All three stages were completed using '*ExploreDTI*' (<http://www.exploredti.com/>). *ExploreDTI* is an academic research software suite created by Alexander Leemans of the PROcessing & VIsualization in Diffusion Imaging (PROVIDI) Lab at Image Sciences Institute – University Medical Center Utrecht, The Netherlands. This program allows complete pre-processing, modelling and computation of diffusion weighted imaging data.

2.6.1. DWI DATA PRE-PROCESSING

Pre-processing is needed to a) convert the diffusion data into the appropriate format and b) correct for known systematic problems inherent in diffusion data. The ultimate goal of pre-processing is to generate reliable and valid diffusion tensor maps (model parameter estimation) that can be used to compute useful diffusion measures (e.g. fractional anisotropy or mean diffusivity). These measures can then be used to generate whole brain tracts. The pre-processing procedure undertaken consisted of the following, and took approximately 1.5 hours per subject;

File Standardisation →

Signal Drift Correction →

Gibbs Ringing Correction →

Generation of *.mat file →

Checking orientation of images →

Corrections

STANDARDISING DIFFUSION INPUT FILES

Unfortunately, due to infrastructural and scanner developments at TCIN, the DWI scans from the scanner were outputted in different formats over the course of the study. Although these different formats contained the same information, they needed to be converted to a useable format, often taking many steps. For use in the *ExploreDTI* program, all source data needed to be converted into a “4D NifTi” file. The worldwide standard for handling and transmitting medical imaging is DICOM (Digital Imaging and Communications in Medicine (DICOM) (Kahn et al., 2007). In this file format, each 2D image slice has its own file, which can result in thousands of separate files in for each individual because of the concentration of our diffusion data collection. As processing such a large number of files is impracticable and highly error prone, these files were converted into more manageable formats (two or three files per subject containing all the relevant diffusion data) by the Phillips scanner. Such formats included ‘*.par/.rec’ (propriety Philips MRI data format from (Philips Healthcare, Best, The Netherlands)), ‘*.bval/.bvec/.nii’ (consisting of b-value/b-vector pair as well as a NifTI (Neuroimaging Informatics Technology Initiative) file containing the MR data) and ‘*.xml/.rec’ (a more recent propriety Philips MRI data format in which the .rec file is replaced by an .xml (Extensible Markup Language) file (Philips Healthcare, Best, The Netherlands))

4D NIFTI

For use in the *ExploreDTI* program, all source data needs to be converted into a “4D NifTi” file. This is essentially a 3D series with an added temporal/diffusion weighted dimension (Jones and Leemans, 2011). This was achieved by using “dcm2nii” tool developed by Chris Rorden (see <http://www.cabiatl.com/micro/mricron/dcm2nii.html> and <http://brainybehavior.com/neuroimaging/2010/05/converting-dicom-images-to-nifti-images/> for further information). This tool allows for easy, reliable and fast conversion of all above file types mentioned above into a dual 4D NifTi (*.nii) and b-matrix text (*.txt) files. The b-matrix is derived from the b-value and the gradient directions (in our case our b-value=1500 s/mm² and 61 gradient directions).

Therefore, at the end of this step, every subject had a NifTi (*.nii) and text (*.txt) file containing all the relevant diffusion data and associated organizational and parameter setting. Of note, each subject also had a T1-weighted image (*.nii file) for the purpose of co-registering the diffusion data to and error correction below.

SIGNAL DRIFT

The combination of diffusion gradients and echo-planar imaging (EPI) readout puts a high load on the system, causing heating effects with continuous use that decrease the scanner's temporal stability (i.e. the signal 'drifts' over the day). This results in an instability of the diffusion data with respect to scanner use, leading to a decrease in global signal intensity (Benner et al., 2006) over the course of the day. This was corrected using a new dedicated plugin in *ExploreDTI* (*plugins* → *correct for signal drift*), which performs linear and quadratic corrections on the data normalizing for any signal drift (Vos et al., 2017).

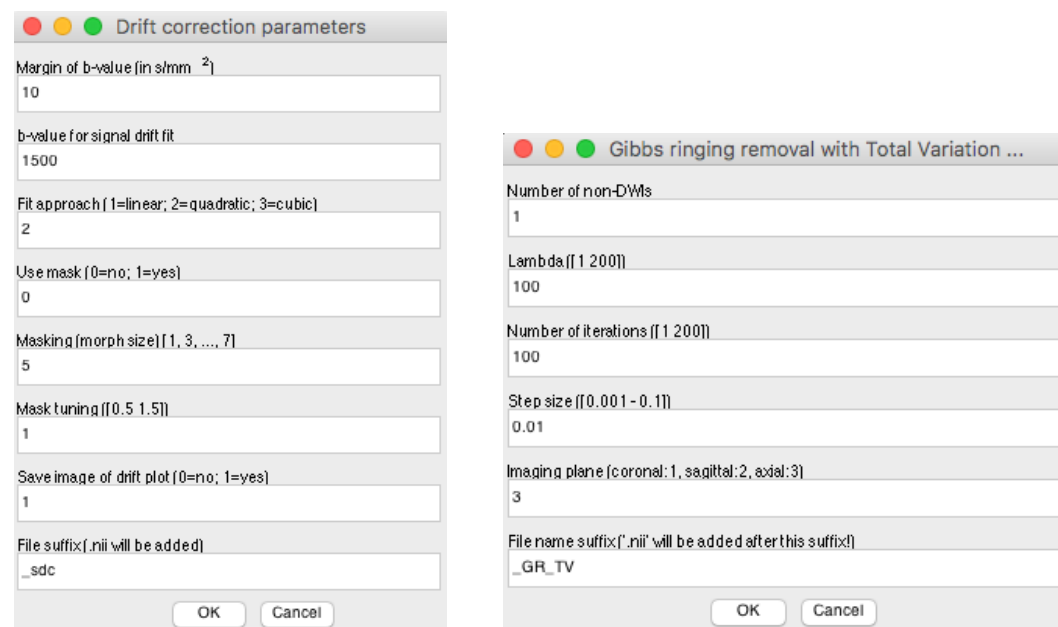


Figure 2.9. Drift correction and Gibbs ringing. Interface and setting for signal drift correction and Gibbs ringing removal.

GIBBS CORRECTION

Gibbs-ringing is a well-known MRI artifact which manifests as spurious oscillations (seen as concentric rings) in the vicinity of sharp image gradients at tissue boundaries (Kellner et al., 2016). This was also corrected using a dedicated plugin in *ExploreDTI* (*plugins* → *TV for Gibbs Ringing in non DWIs 4D*.nii*). This tool applies an arithmetical total-variation computation (Clarkson and Adams, 1933). This is a mathematical correction measure applied across the data that penalises for noise at the sharp boundaries within the image (Perrone et al., 2015) while preserving the other useful features of the data.

CONVERTING *.NII/TXT TO *.MAT

ExploreDTI is a MATLAB based program. For use in the MATLAB environment, all diffusion data needed to be converted from standard diffusion format to a MATLAB readable file. By converting files from DWI (*.nii) and associated B-matrices (*.txt) files to a single *.mat file, allowed *ExploreDTI* to condense all the relevant diffusion information into a single file for computational manipulation and visualization. This was achieved relatively simply through a *ExploreDTI* data conversion tool (*calculate DTI .mat file* → *convert raw data to '*.DTI.mat'*) (see figure X) and provides the basic usable file formation for all preprocessing and computational steps to follow. Spatial and gradient orientations of the diffusion data (i.e. anterior-posterior, right-left, inferior-superior) can be corrected during in this step.

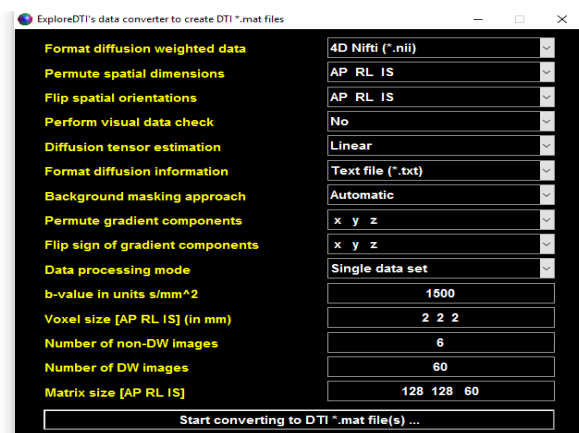


Figure 2.10. Converting to *.MAT. Interface and settings for converting *.nii and *.txt files to *.MAT files. Note in particular the Flip/permute spatial and gradient components. These can be adjusted to change the orientation of the image or diffusion directions as needed.

*CHECKING ORIENTATION OF GENERATED *.MAT FILE*

At this stage, each newly generated *.mat file needed to be checked for correct orientation. This was achieved by checking directionality of the 'glyphs' in specific areas of the brain. A tensor glyph is a graphical visualization tool that allows visualization of the overall direction (or vector) of diffusion at each voxel (typically referred to as the diffusion tensor). We know from neuroanatomical studies that certain large white matter tracts of the brain have a specific directionality (e.g. corpus callosum axons predominately travel from left to right). By using this information we can check the orientation of the *.mat file by reviewing the glyph directions in these areas. This was achieved in *ExploreDTI* by loading up the newly generated *.mat file, locating an appropriate region of interest (ROI) and using the glyph plugin (*menu → draw → glyph → object*). The three tracts used to check glyph orientation were; a) corpus callosum (across; left to right), b) corona radiata (vertical; inferior to superior) and c) superior longitudinal fasciculus (lengthways; anterior to posterior) (see Figure 2.11. a-d). If the glyph directionality in these areas was obviously incorrect (e.g. glyphs in the corpus callosum pointing in an anteroposterior direction instead of in a left to right direction), the *.mat file was corrected and recalculated by adjusting the gradient orientations as needed (see previous step and specifically Figure 2.10.). This fundamental step ensures the correct later propagation of the diffusion tensors used to form the deterministic streamlines and construct the characteristic fibre tracts. Incorrect orientations of the diffusion images results in nonsense tracts. About 20% of the time, these diffusion files needed gradient orientation adjustment.

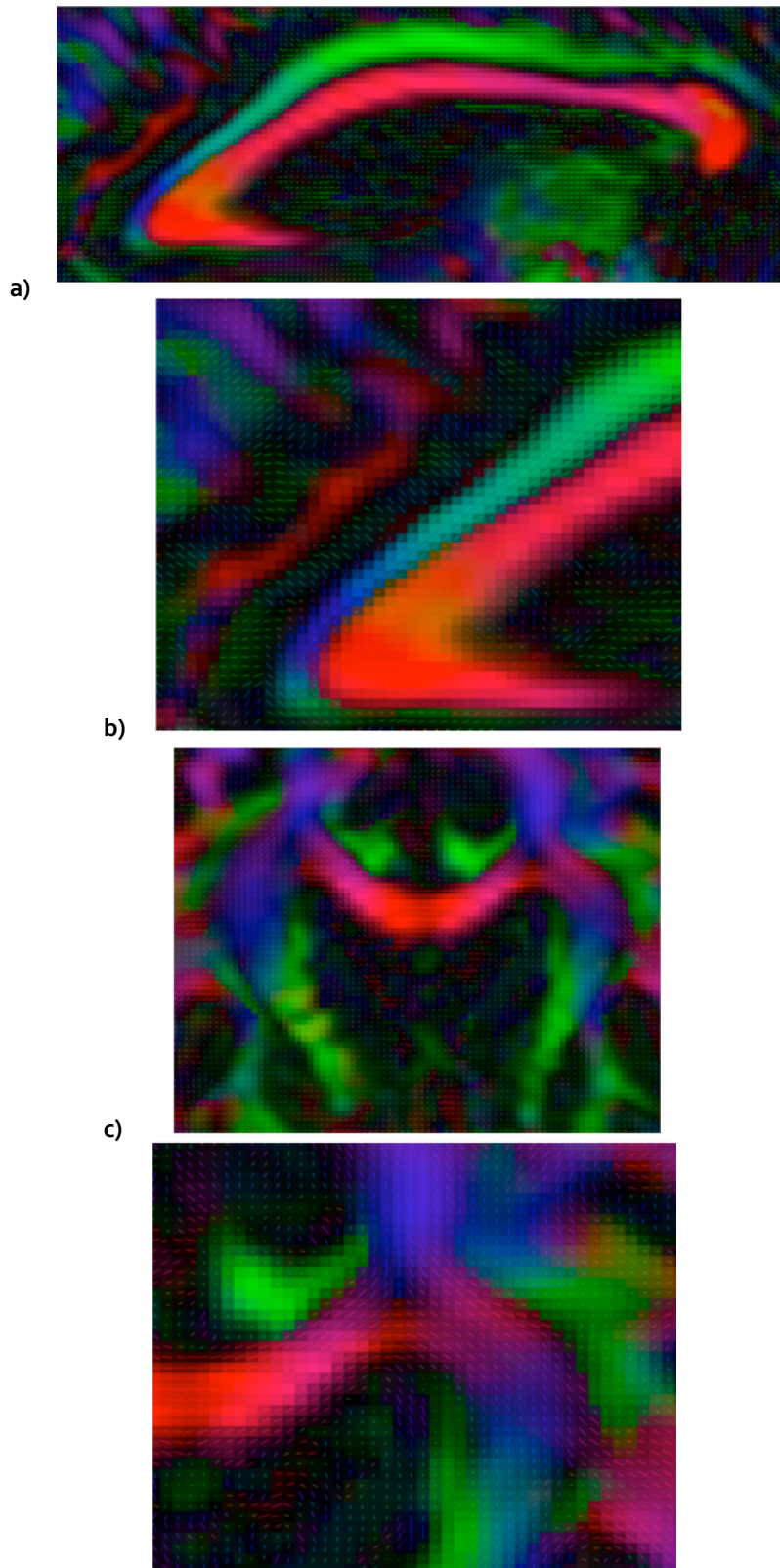


Figure 2.11. (a-d) Checking for glyph orientation. Glyphs need to be checked according to known neuroanatomical characteristics of standard tracts, such as the corpus callosum. a) sagittal view of corpus callosum; b) close up of genu of corpus callosum; c) coronal view of corpus callosum; d) close of body of corpus callosum.

CORRECTING FOR EDDY CURRENTS AND HEAD MOTION

MRI acquisition is subject to many artefacts that can affect data acquisition, leading to problems with parameter estimation (i.e. the diffusion tensor maps) and subsequent computed measures (e.g. fractional anisotropy maps). As such artefact correction is one of the most crucial aspects of post-acquisition processing (Soares et al., 2013). The two main artefacts intrinsic to diffusion MRI acquisitions that may destroy voxel-wise correspondence across all the DWIs are eddy current distortions and head motion (Rohde et al., 2004, Mohammadi et al., 2010).

Eddy currents arise in DWI imaging due to the rapid switching of the large gradient pulses that occur during high field diffusion imaging (Tournier et al., 2011). These large swings in magnetic gradients can induce currents in the various conducting surfaces of the MRI scanner. These currents can cause image distortions (seen as an overall shift and shear of the image as well as image contraction in severe cases).

DWI is particularly sensitive to motion, due to phase shifts induced a) microscopically by diffusion-driven water molecular displacements, and b) macroscopically by cardiac pulsation, breathing and head motion. This sensitivity increases with the intensity and duration of gradient pulses (characterized by the b-value) (Le Bihan et al., 2001). Unfortunately, the process of DWI acquisition also generates a considerable amount of table movement due to the rapid shifting of gradient pulses.

Correcting for both artefacts requires careful registration to the non-diffusion weighted b- image. In practice this means that the diffusion image is adjusted (transformed) to match the T1 image. As the DW image contrast is orientation as well as spatially dependent, the B-matrix (which contains the diffusion gradient information) must also be correctly adjusted so the orientation is correctly preserved.

Eddy currents can be corrected with an affine⁴ registration to the b-o image and movement or head motion is corrected with a rigid body registration to bo image (Soares et al., 2013). Cardiac pulsation can be further corrected with adaptive acquisition sequence timing parameter adjustment to synchronize the cardiac pulse period with the data acquisition and hence minimize the influence of this movement on the data. Cardiac pulsation adjustments can be very costly in terms of acquisition time and hence were not included in this study. The correction was performed and achieved in *ExploreDTI* through using the "Correct for Subject Motion and EC" plugin and adjusting the settings as needed accordingly performs this process without the need to include such time penalties during acquisition.

CORRECTING FOR EPI DEFORMATIONS

Another artefact that degrades diffusion MRI data quality is due to the so-called *susceptibility-induced off-resonance fields*, which cause non-linear geometric image deformations. These are a common problem with the echo planar imaging acquisition used in this study. Acquiring additional data during the scan by reversing the polarity of the phase-encoding direction can minimize this effect. However, the extra time required and comfort constraints prevented us from doing this. Hence, the EPI deformations were corrected in *ExploreDTI* using a specialized tool, incorporated into the previous corrective tool for head motion and eddy currents, which allowed for affine registration (see footnote) and warping of the DWI to the acquired T₁ image (this can be achieved because the T₁ image isn't susceptible to the same inhomogeneities in the diffusion echo planar imaging sequence) (Irfanoglu et al., 2012).

⁴ In rigid registration, only the position of the DWI image relative to the bo image changes (allowing for 6 degrees of freedom – translation along the x, y, z axes as well as rotation around the x,y,z axes). Affine registration allows change of position *and* geometry of the DWI image (translation and rotation as above, but also including stretching along the x, y, z axes and shearing/skewing the xy, yz and xz planes). This allows up to 12 degrees of freedom.

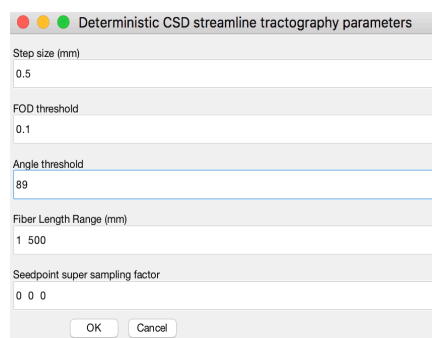
2.6.2. WHOLE BRAIN TRACTOGRAPHY

ExploreDTI permits deterministic and probabilistic fibre tractography, clustering of fibre tracts and data quality assessment tools as well as Constrained Spherical Deconvolution (CSD)-based tractography. Deterministic tractography as a technique to visualise tracts is an experimental method that is subject to some technical challenges (Jones, 2008). There are known issues involving closely aligned curving and kissing tracts. Both the fornix and the stria terminalis are highly curved and turn 180° in a relatively tight space. This was addressed by reconstructing the fornix-stria using high angle (89°), small step-size (0.5 mm) whole brain tracts. These tracts lie deep within the centre of the brain, surrounded by and touching other white matter bundles. CSD was chosen as the most appropriate tractography technique due to its superior ability at detecting complex crossing, hugging and kissing fibres in a recent analysis of fibre estimation approaches (Wilkins et al., 2015). CSD enables accurate reconstruction of local fibre orientation in crossing fibre regions compared to the more typical tensor based or probabilistic methods (Jeurissen et al., 2011). This algorithm has been shown to overcome some of the known difficulties and shortcomings of the diffusion tensor model and has the ability to model regions with complex fibre orientations such as compound or kissing fibres within a single voxel, allowing more convoluted tracts to be accurately explored. By default, *ExploreDTI* uses a recursive calibration of the response function as the method of CSD calculation (Tax et al., 2014).

Tractography of the whole brain was generated in *ExploreDTI* using CSD modelling of all potential deterministic streamlines throughout the entire brain according to the following parameters; seed point resolution was set at 2 x 2 x 2 mm with a step size of 0.5mm, the FOD (Fibre Orientation Distribution) threshold was set to 0.1, and a maximum angle threshold set to 89 degrees (See Figure 2.13.). This means that the CSD tractography algorithm, which propagated in 0.5 mm steps along this direction, estimated the principle diffusion orientation at each point. At each new location the fibre orientations were estimated again before the tracking moved a further 0.5 mm along the direction that subtended the smallest angle to the current trajectory. A

trajectory was followed through the data until the scaled height of the fibre orientation density function peak dropped below 0.1, or the direction of the pathway changed through an angle of more than 89°. Other studies have used various combinations of step sizes of 0.5mm (Christiansen et al., 2016, Kehoe et al., 2015, Metzler-Baddeley et al., 2013) or 1 mm (Tang et al., 2017, Postans et al., 2014) and angle thresholds of 30 (Postans et al., 2014) 45 (Christiansen et al., 2016, Tang et al., 2017) and 60 (Metzler-Baddeley et al., 2013) degrees for reconstruction of the fornix. The one previous study that investigated the stria terminalis (using probabilistic rather than deterministic tractography) used an angle threshold of 60° but did not report a step size. During my preliminary tests, I discovered that both decreasing the step size and increasing the angle threshold to the maximum possible (89 degrees) resulted in much more robust fornix and stria terminalis tracts. This is because both of these tracts are highly curved, turning an arc of 270 degrees over their course. By decreasing the step size and increasing the angle threshold I generated a maximum number of potential streamlines along these tracts, however at a cost of increasing computing resources. This stage took approximately 1 hour per subject and generated a large (between 4 and 8 gigabytes) CSD whole brain tractography file per subject.

Following this step, all files were randomized using a random number generator (<https://stattrek.com/statistics/random-number-generator.aspx>) so the individuals doing the manual tractography and fornix and stria extraction were blind to the age, sex and group of each subject. This randomization was undertaken to prevent bias.



Deterministic CSD streamline tractography parameters	
Step size (mm)	0.5
FOD threshold	0.1
Angle threshold	89
Fiber Length Range (mm)	1 500
Seedpoint super sampling factor	0 0 0
OK Cancel	

Figure 2.12. Constrained Spherical Deconvolution. Interface and settings for constrained spherical deconvolution. Note step size of 0.5 mm and the angle threshold of 89 degrees.

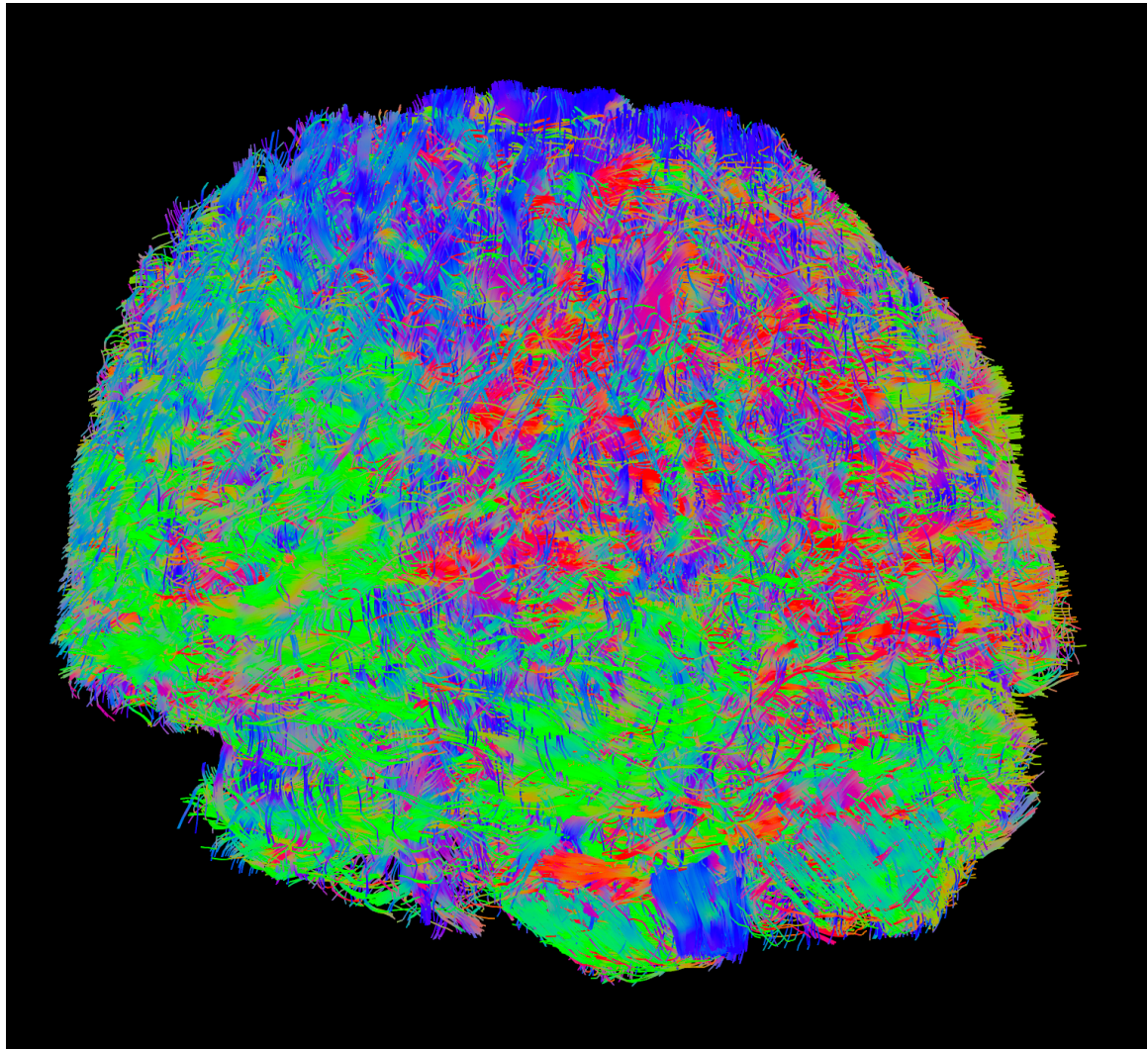


Figure 2.13. Example of full brain tractography. All calculated tracts in the brain are shown. From this rather complex structure, specific tracts can be 'dissected' using known neuroanatomical markers. The different colours contain directional information related to the FA along the tracts. The top of the spinal cord can be clear seen as a solid blue structure near the base. FA; fractional anisotropy.

2.7. FORNIX AND STRIA TERMINALIS 'DISSECTION'

The fornix and stria terminalis closely follow each other in a loop like structure around the thalamus. It is difficult to differentiate both these structures from each other using standard tractographic techniques. In fact, much tractography research investigating the fornix actually investigates the fornix-stria terminalis combination (fornix-stria), but does not refer to this fact (see Chapter 1.5. and Chapter 1.6.). However, it has been shown that it is possible to separate the stria terminalis from the fornix using probabilistic tractography study (Kamali et al., 2015a). However, this technique is time and computer resource consuming and not anatomically driven. Therefore, using probabilistic tractography, the stria terminalis streamlines may be easily conflated or confused with those of the fornix. Thus, I designed a method using the amygdala to essentially subtract the stria terminalis from the fornix-stria combination. Reversal of the method renders a fornix.

Following consultation with the Department of Anatomy at Trinity College Dublin, a Boolean logic protocol was designed to isolate the fornix-stria combination using AND and OR gates within *ExploreDTI*. Emphasis was given towards gates that: (a) included the maximum possible number of fornix –stria streamlines; (b) were positioned around landmarks clearly identifiable on DWI or T1 images; and (c) that would consistently capture at least some section of the fornix-stria.

This extraction protocol involved the use of six ROI gates. This step was undertaken by two independent raters Anna Martin and Tom Drago (medical students, Trinity College Dublin) who were trained by me to implement the protocol on a training data consisting of 15 processed diffusion images. During training, the gates and tract outputs of both raters were checked and compared to insure as close and alignment as possible. Outputs were compared between raters after every two tracts and a consensus between both raters and me resulted in adjustment of the gates if needed. After ten or so tracts, the raters had near similar outputs in terms of gate placement,

shape of tract, tract length, tract volume and number of streamlines. Following training, the raters both worked independent from each other on the entire dataset, blind to the group (control or depressed), age and sex of the subjects.

FORNIX-STRIA PROTOCOL

OR (SEED) Gate

A coronal slice was taken where the fornix descends inferiorly as the columns. The slice was taken posterior to the anterior commissure and postcommissural fibres. The fornix appears as a light green/blue bullet shape in this slice. The OR gate was drawn halfway through the body of the corpus callosum horizontally, descending along the corona radiata through the posterior limb of the internal capsule as far down as the lenticular fasciculus. The gate was then drawn straight across and mirrored on the opposite side (Figure 2.14.).

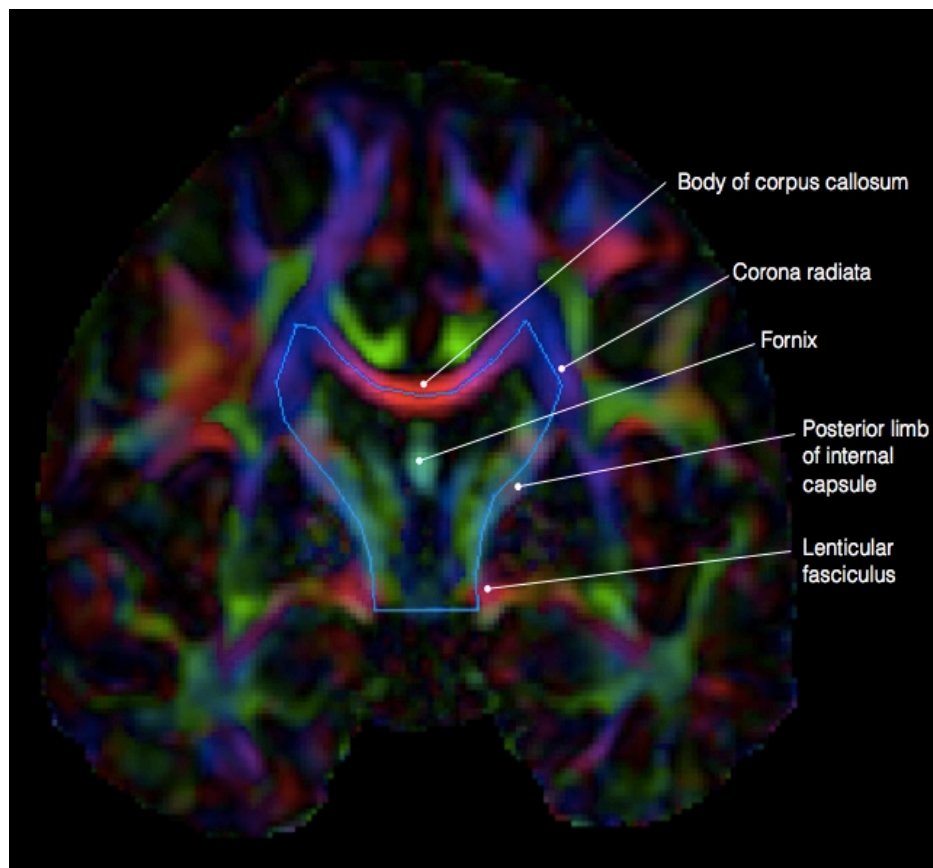


Figure 2.14. Initial OR gate. Coronal slice with OR gate in blue. See text for details of protocol.

AND Gate

An axial slice was taken one step below the level of the anterior commissure so the fibres (seen in red) do not join in the midline. The AND gate was drawn starting midway through the blue portion of the cerebral peduncle. The gate was then continued laterally out to the inferior longitudinal fasciculus (ILF) and followed the outer edge of the ILF until it was past the splenium of the corpus callosum (confirmed in the sagittal view). It was then drawn straight across and continued up the outer edge of the ILF on the other side of the midline, across to the cerebral peduncle. The crura of the fornix are seen in green in this slice; it should be ensured that they are well within this gate (Figure 2.15.).

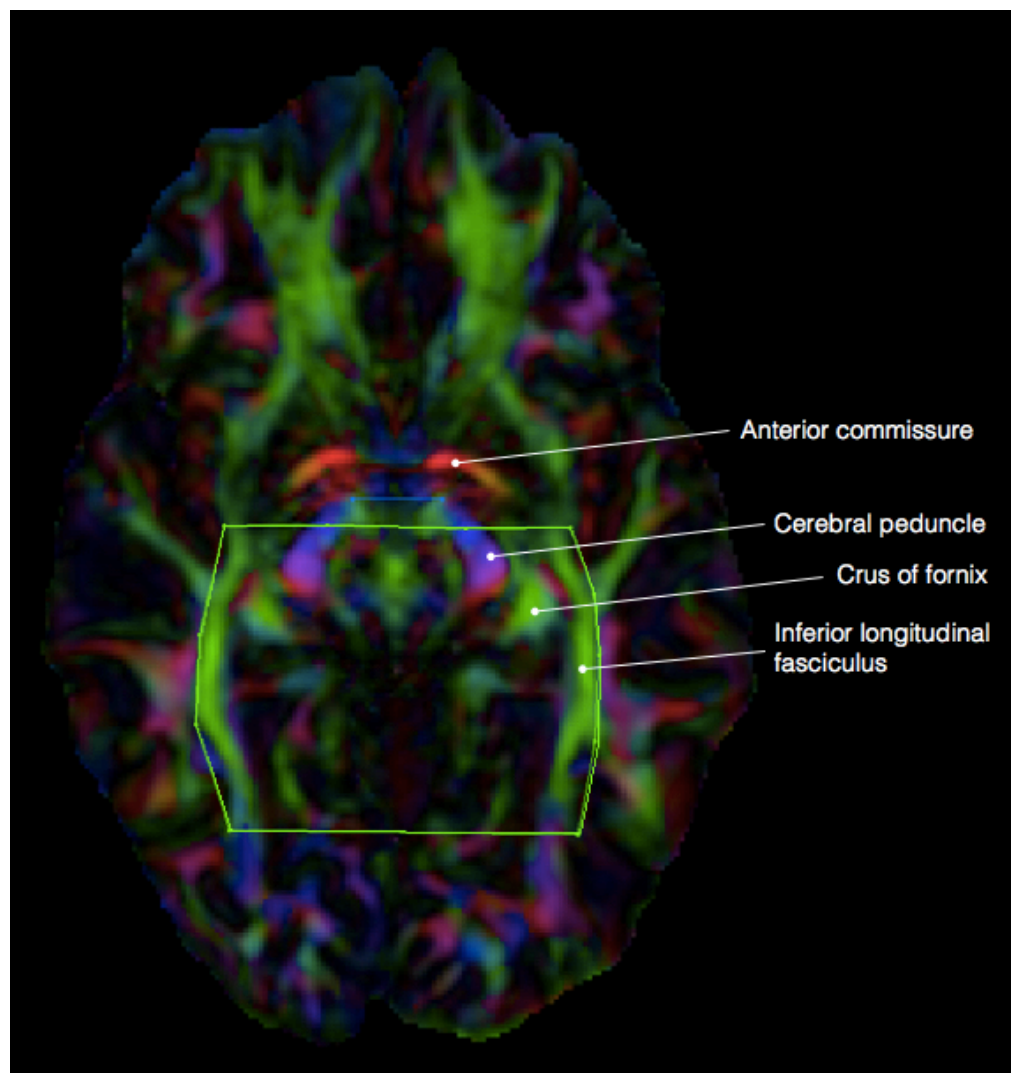


Figure 2.15. Initial AND gate. Axial slice with AND gate in green. See text for details of protocol.

NOT₁ Gate

A coronal slice was taken through the posterior tip of the genu of the corpus callosum. The NOT gate was drawn around the full slice.

NOT₂ Gate

An axial slice was taken midway through the body of the corpus callosum. The NOT gate was drawn around the full slice.

NOT₃ Gate

An axial slice was taken through the brainstem where the temporal lobes are partially joined. The NOT gate was drawn around the brainstem but not around the temporal lobes as some fornix fibres extend into this area.

After the above gates were placed, the fibre tracts were loaded, analysed and drawn, giving the robust structure of the fornix. This was used as a guide for the placement of the final gate.

NOT₄ Gate

A coronal slice was taken just behind the curve of the crura of the fornix, making sure not to cut off any fibres that followed the tract of the fornix. The NOT gate was drawn around the full slice.

The placement of all the gates, including the four NOT gates in the sagittal view of the brain without (Fig. 2.16 A) and with (Fig. 2.16 B) the final fornix-stria are shown.

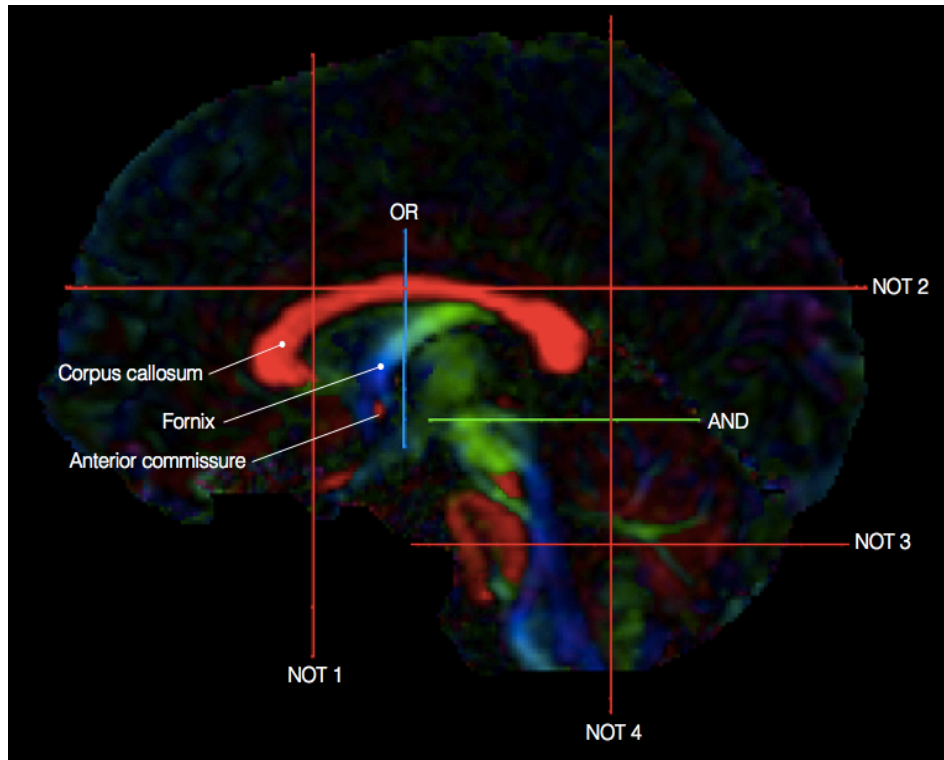
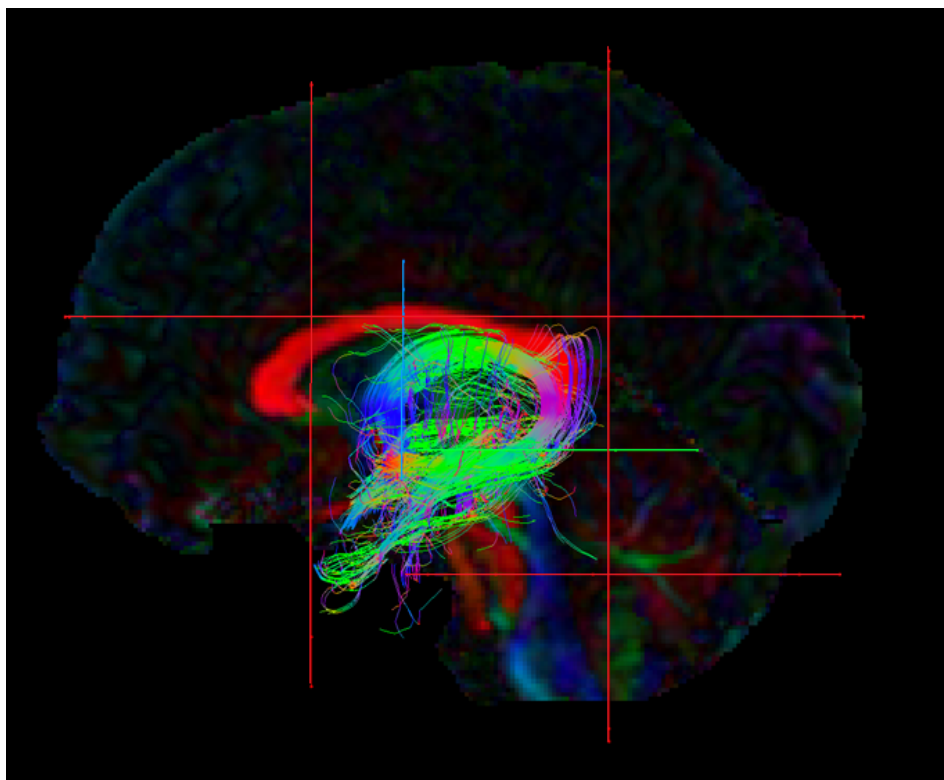
A**B**

Figure 2.16. Sagittal view showing all gates. OR gate (blue), AND gate (green) and 4 NOT gates (red) without (A) and with (B) the rendered fornix. The fornix appears as the arching structure under the red corpus callosum that turns from green (body) to blue (columns). Following on from the columns, the blue postcommissural and precommissural fibres diverge at the anterior commissure (red).

The tracts were then deleted, analysed and drawn again to render the final fornix-stria composite. For each subject, the gates were assessed and adjusted if required to ensure all fornix fibres were being encompassed by the OR and AND gates, and no fibres were being cut off unnecessarily (known as clipping). The ROIs were saved and the tracts were saved as a *.mat file.

Every fornix-stria is different and an amount of variation in streamline density was allowed as normal. However, if a rater was unhappy with a rendered tract for any reason, I checked the tract and gates manually before continuing. Reasons for this mostly included misplaced gates, a large number of extraneous streamlines not consistent with the fornix-stria (usually due to overly inclusive AND and NOT gates) and a thin or lopsided fornix-stria (usually due to overly inclusive NOT gates). Each tract from each rater was also checked by myself at the end of the entire process and compared visually to the same tract from the other rater. I edited any obvious discrepancies accordingly.

This process was repeated for all 60 subjects (30 depressed and 30 controls) independently by two different trained raters (Rater 1 and Rater 2). A comparison of reliability between each independent rater was performed using interclass correlation coefficient (ICC) analysis (Johnson and Kotz, 2011) for the following measurements; dimensional measures; SM length and tract volume; standard diffusion metrics; FA, MD, AD and RD. These are reported in Appendix 4.

2.7.1. EXTRACTING THE STRIA TERMINALIS FROM THE FORNIX-STRIA

The Stria Terminalis is the primary output from the amygdala (Gloor, 1978). Using the 'Calculate from folder of masks' option an amygdala volume mask (previously generated from *FreeSurfer*) was used as an AND gate with the fornix-stria tracts. This was repeated for each side (i.e. left and right amygdala) and for each subject. This generated a group of streamlines that only originated from the amygdala.

The next step required that streamlines not associated with the stria terminalis be removed. Cleanup was achieved with two raters (medical students Anurag Nasa and Caoimhe Gaughan) and a neuroanatomist from the Department of Anatomy at Trinity College Dublin examining every subject individually, collectively excluding streamlines not considered stria terminalis fibres using NOT gates. Criteria for exclusion included streamlines deviating from the known arch and loop shape of the stria terminalis and streamlines consistent with known adjacent tracts. To address potential bias in the same subject between raters, the neuroanatomist was blind to the identity of each subject. These NOT gates were variable according to each subject and depended on the individual neuroanatomy of the region. However, commonly excluded streamlines included fibres from the body of the fornix, the anterior commissure, the median forebrain bundle, stria medullaris and amygdalofugal pathway, and various inter-thalamic connections. A second neuroanatomist reviewed a random subsample of 25 tracts from both raters to check face validity of the stria terminalis (looking at start and end points, the arch shape, the tract's relationship to other structures on MRI images (thalamus, fornix, anterior/posterior commissures and third ventricle) as well as the overall general impression of the tract. The second neuroanatomist also checked both raters' tracts simultaneously to investigate if they were occupying approximately the same space. This was considered a sufficient subsample for quality control.

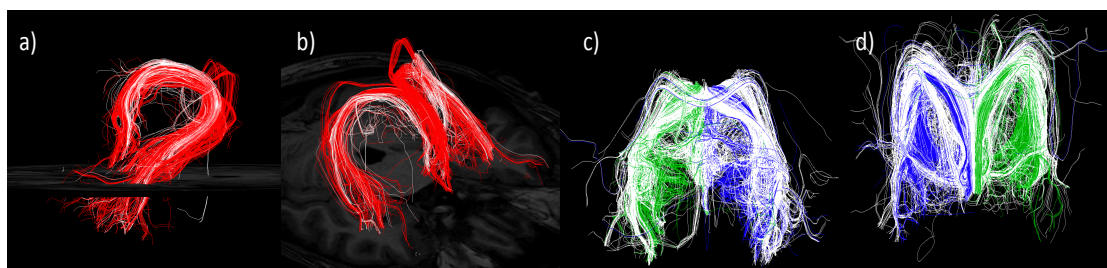


Figure 2.17. (a-d) Fornix-stria combinations. a) & b) showing left sagittal and oblique frontal views respectively. The fornix is coloured red and the stria terminalis is white. c) and d) showing a frontal coronal and superior axial views respectively. The fornix is coloured green and the stria terminalis is blue.

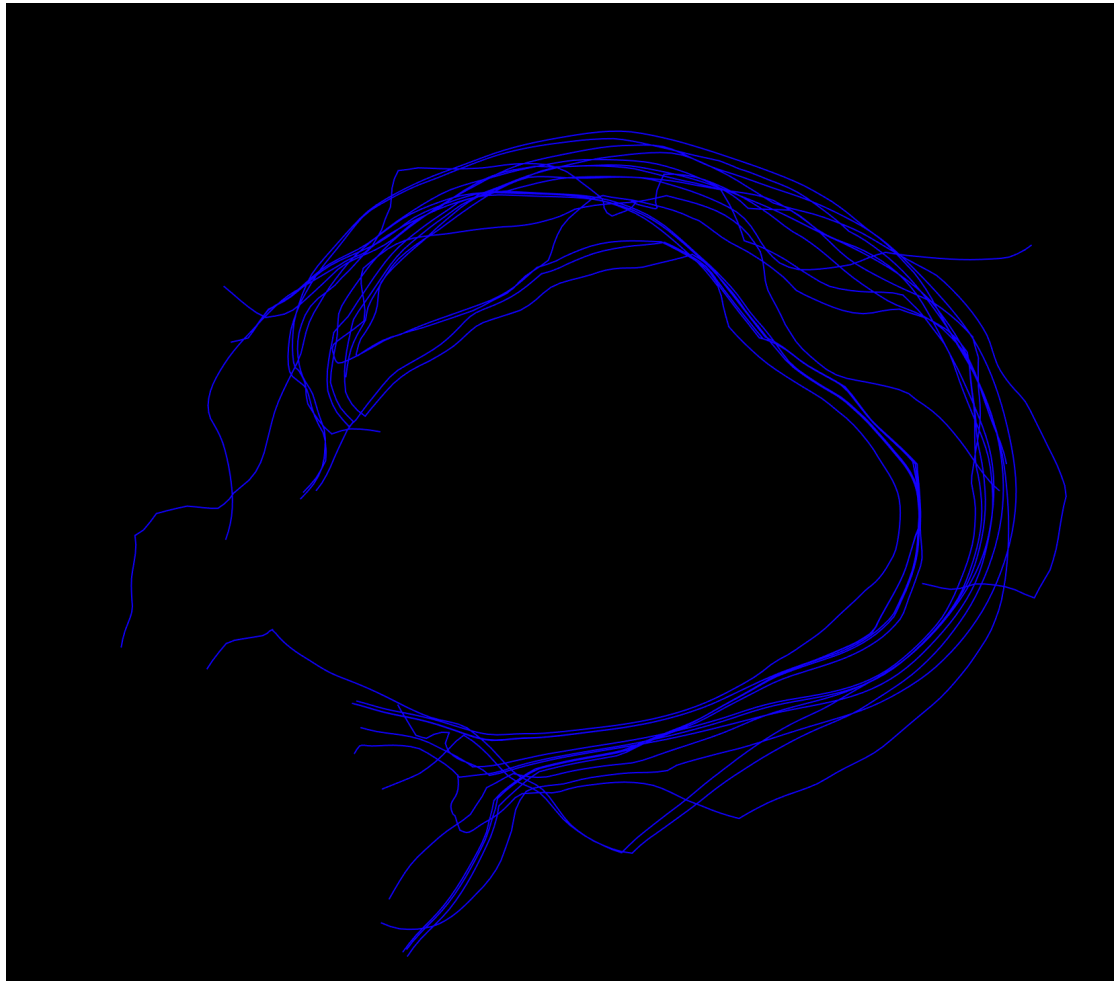


Figure 2.18. Completed but poorly rendered stria. An example of a poorly rendered completed stria. Compare to the number of streamline and density of streamlines of completed stria in Chapter 6, (Figure 6.3)

2.7.2. EXTRACTING THE FORNIX FROM THE FORNIX-STRIA

The fornix is the primary output from the hippocampus (Thomas et al., 2011). Using the 'Calculate tracts from folder of masks' option both bilateral amygdala volume masks (previously generated from *FreeSurfer*) were used as combined NOT gates with the fornix-stria tracts. This generated a group of streamlines from the fornix-stria that excluded the stria terminalis bilaterally. Following this, a simple AND gate around the crus of each fornix generated the left and right fornices.

Cleanup followed a similar procedure to the stria cleanup with the same two raters and neuroanatomist using NOT gates. Criteria for exclusion included streamlines

deviating from the known arch and loop shape of the fornix and streamlines consistent with known adjacent tracts. Commonly excluded streamlines included fibres from the hippocampal commissure, lyra, anterior commissure, the median forebrain bundle, stria medullaris and amygdalofugal pathway and various inter-thalamic connections. A similar quality check from a neuroanatomist was used as above.

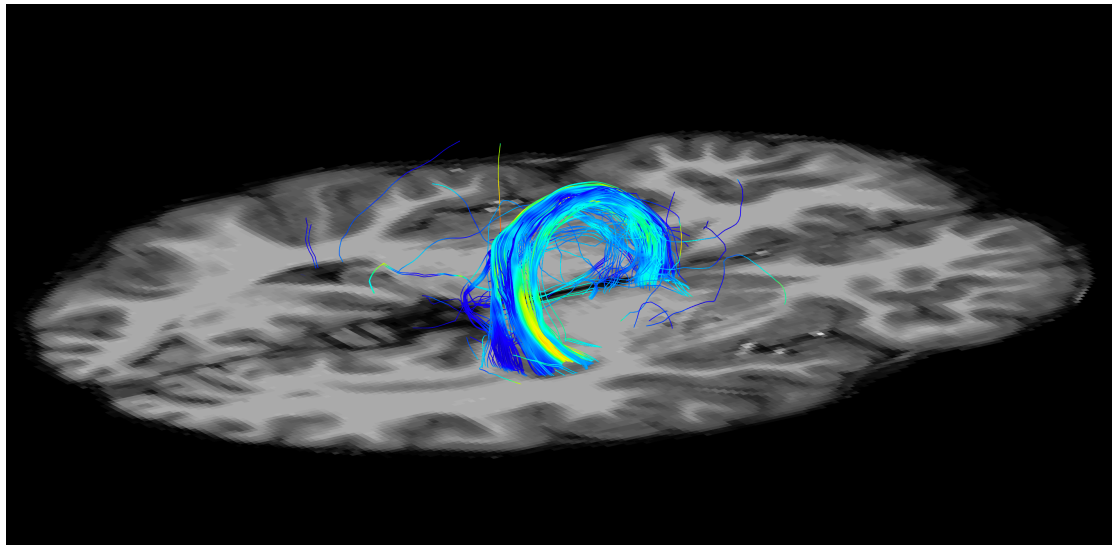


Figure 2.19. Completed Fornix. Almost completed right fornix on T1 axial slice. Stray streamlines evident above will be removed prior to analysis

2.7.3. TRACT METRICS

Tract statistics including dimensions (length and volume), standard diffusion measures such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) and Westin diffusion measures [spherical diffusion (CS), linear diffusion (CL) and planar diffusion (CP)] were calculated on each whole-tract using *ExploreDTI's* built in tract statistics plugin (*Plugins → Convert → Info of tract *.mat file(s) to *.txt*). Westin measures can be used to give more information about processes occurring in the tracts.

Along-tract measures were also calculated using *ExploreDTI's* along-tract analysis function. Basic tensor metrics were calculated using the following equations (Feldman et al., 2010):

Axial diffusivity

$$\lambda_1$$

Radial diffusivity

$$\frac{\lambda_2 + \lambda_3}{2}$$

Mean diffusivity

$$\frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

Fractional anisotropy

$$\sqrt{\frac{1}{2} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}}{\sqrt{(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)^2}}}$$

2.8. CORTISOL MEASURES

Salivary cortisol was measured in a subsample of participants. All subjects were offered to partake in salivary cortisol collection, however a significant proportion declined, and another significant proportion failed to engage fully with sample collection or return.

SALIVA SAMPLE COLLECTION AND STORAGE

Saliva samples were collected in Salivette® tubes (Sarstedt) by the study participant at three time points after waking (0, 30, and 60 minutes). Sample collection always took place on a 'normal' weekday. This is because changes in daily routines are known to impact cortisol responses (Dedovic and Ngiam, 2015). The completed sets of samples were returned in person or by post as soon as possible. Once the completed set of saliva samples was received, they were centrifuged for 10 minutes at 3,000rpm at room temperature. The insert was then removed discarded from the salivette. The saliva was aliquoted into 500µL samples and stored in microtubules at - 80°C until bulk analyses were carried out.

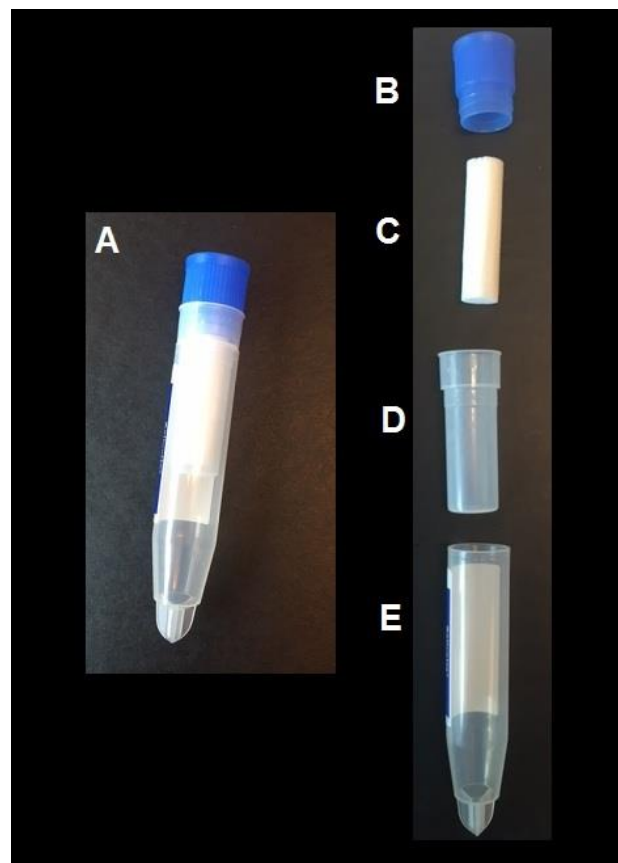


Figure 2.20. Example of Salivette® tubes. At the appropriate times, participants were instructed to remove the cap (B) and the swab (C), then place the swab in their mouth and under their tongue for 30 seconds or until the swab was saturated. The swab was then replaced into the inner tube (D). Centrifuging the Salivettes extracted the saliva, through the inner filter and into the outer tube (E). Finally, the saliva was aliquoted from the outer tube into microtubules and subsequently frozen.

LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY FOR SALIVARY CORTISOL

Liquid Chromatography – Mass Spectrometry (LC-MS) was performed at the University of Manchester in the Department of Clinical Biochemistry. Members of Professor Brian Keevil's laboratory at the University Hospital of South Manchester conducted this work. Even though I was not directly involved in carrying out this laboratory analysis, I describe the method briefly here. The following protocol description has been adapted from LJ Owen et. al. 2010 which describes these methods.

SAMPLE PREPARATION FOR LC-MS

Stock solutions were prepared by dissolving hydrocortisone (Sigma, UK) in methanol (Sigma, UK) and were stored at -20°C. A separate stock solution was prepared for standards and quality controls (QCs), both 10 mg/ml. Working standards and QCs were prepared by diluting the stock solution with phosphate buffered saline (PBS) pH 7.4 (Sigma, UK) to give concentrations of 0, 3.4, 17, 34, 67 and 100 nM and 5.6, 28 and 56 nM, respectively. Aliquots of these were stored at -30°C. Deuterated cortisol (D₂) was used as internal standard (CDN Isotopes, Canada) at a working concentration of 30 mg/L in methanol. Standard, QC or sample (200 µl) was manually pipetted directly to the well of a 96-deep-well block (Abgene, UK). To this, 25 µl of working strength internal standard was added. The block was thermosealed (Abgene, UK) and vortexed for one minute, then centrifuged at 1500g for 10 minutes. Following centrifugation, the block was transferred directly to the autosampler for analysis; 50 µl of sample was injected into the liquid chromatography (LC) system using partial loop mode (Owen et al., 2010).

CHROMATOGRAPHY

A Shimadzu Prominence LC system (Shimadzu, Milton Keynes, UK) was used for chromatography. The mobile phases utilized were: A, water with 2 mmol/L ammonium acetate (Sigma, Poole, UK) and 0.1% formic acid (VWR International Ltd, Leicestershire, UK); and B, methanol (LC-MS grade, Riedel-de Haen, Hanover, Germany) with 2 mmol/L ammonium acetate and 0.1% formic acid. The sample was

injected onto a Phenomenex C8 4 x 2 mm guard cartridge (Phenomenex, Macclesfield, UK) using 10% mobile phase B at a flow rate of 1 ml/min for 0.6 min. The eluate from the guard cartridge during this time was diverted to waste to allow sample cleanup and removal of saliva matrix before elution onto the analytical column.

After 0.6 min, the mobile phase composition was stepped up to 60% B and flow rate reduced to 0.4 ml/min to elute the cortisol from the guard cartridge. The eluate was directed onto the analytical column, a Phenomenex 30 x 3 mm 4 μ m Synergy Hydro-RP C18. The flow was maintained at 60% B until 2.5 min when it was stepped up to 100% B for 0.7 min before returning to 60% B for the final 0.8 min. The small delay between injections (40 s) on this system was enough to re-equilibrate the guard cartridge with 10% B while maintaining the analytical column at 60% due to the initial divert to waste. Both guard and analytical columns were maintained at ambient temperature (Owen et al., 2010).

MASS SPECTROMETRY

The eluate was injected directly into a Quattro MicroTM tandem mass spectrometer (Waters, Manchester, UK) with a Z spray ion source. MassLynx NT 3.5 software was used for system control, the MassLynx QuanLynx programme allowed data processing. This software used the height of the detected peaks, 1/x weighting and linear least-squares regression to produce a standard curve. The 1/x weighting gives greater accuracy at lower concentrations (Owen et al., 2010). The mass spectrometer was operated in electrospray positive mode, the capillary was maintained at 1 kV and the source temperature was 140°C. The desolvation temperature and gas flow were 400 C and 1000 L/h, respectively. The most abundant transitions identified for cortisol and D2-cortisol using 1 mg/L tuning solutions of each compound in 50% mobile phases were m/z 363.2 > 121.1 and 365.1 > 122.2, respectively. The cone and collision energies were 26 V and 22 eV for cortisol and 22 V and 22 eV for D2-cortisol. A confirmatory ion transition for cortisol was monitored at m/z 363.2 > 97.0. Transitions

were monitored in multiple reaction-monitoring (MRM) mode, with a dwell time of 0.15 s (Owen et al., 2010). The entire LC-MS procedure was performed at the Department of Clinical Biochemistry University Hospital of South Manchester, University of Manchester, UK through collaboration with Professor Brian Keevil.

2.8.1. CALCULATION OF CORTISOL AWAKENING RESPONSE PARAMETERS

Data cleaning was carried out by eliminating values that were above or below two standard deviations from the mean; however there were no outliers found in the morning time point LCMS data. Cortisol awakening response (CAR) was calculated using the Area Under the Curve (AUC_i) with respect to increase from baseline and also the AUC_g (AUC with respect to ground) (Fekedulegn et al., 2007). The equation is as follows:

$$[(T_0 \text{ cortisol} + T_{30} \text{ cortisol}) / 2 \times \text{time difference} + (T_{30} \text{ cortisol} + T_{60} \text{ cortisol}) / 2 \times \text{time difference}] - [T_0 \text{ cortisol} \times \text{total time difference}]$$

Where T₀, T₃₀ and T₆₀ are the salivary cortisol concentrations 0, 30 and 60 minutes after wakening.

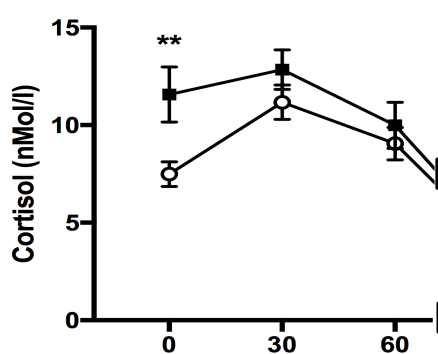


Figure 2.21. Cortisol awakening response. Note the increased cortisol secretion 30 minutes after waking, followed by a slight reduction. The squares refer to the CAR in a MDD patient, and the circles to the CAR in a control participant. CAR, cortisol awakening response.

2.9. STATISTICAL ANALYSIS

In this thesis, demographic details were compared on each cohort (i.e. full eighty-three patients versus eighty controls for hippocampal and amygdala measures, and thirty patients versus thirty controls for tract measures). The following structure specific tests were performed; hierarchical regression was performed on the hippocampal and amygdala results only, hippocampal measures only were analysed with respect to FPD and RD groups; amygdala measures only were analysed with respect to right-left asymmetry; amygdala measures only were correlated with the cortisol awakening response.

2.9.1 DEMOGRAPHIC STATISTICAL ANALYSIS

Demographic information was analysed using the statistical analysis program SPSS 24 (<https://www.ibm.com/analytics/us/en/technology/spss/>). Independent t-tests (Student, 1908) were performed to examine between-group differences in age, and HAM-D for controls versus FPD, controls versus RD and FPD versus RD. Chi-square tests (Pearson, 1900) for independence were performed to examine differences within the proportion representation of males and females between the depressed and control groups.

2.9.2 HIPPOCAMPUS AND AMYGDALA STATISTICAL ANALYSIS

All extracted subfield volumes (both amygdala and hippocampus) were systematically inspected visually and volumetric measures were then exported to SPSS24 (<https://www.ibm.com/analytics/us/en/technology/spss/>). Outliers were removed to ensure that all data fell within the 95% confidence intervals. The systematic removal of outliers ensured that extreme or atypical data points did not influence the results. The depressed group was analysed as a complete group and subsequently as distinct FPD and RD sub-groups for hippocampal data and just the complete group for the amygdala data.

PRIMARY ANOVA (SUBSTRUCTURES)

A primary mixed-model analysis of variance (ANOVA) was used to investigate group-wise differences in substructure volumes across all substructures and hemisphere (left and right) of the hippocampi and amygdalae. For the hippocampus this analysis was performed with the 9 substructure volumes and hemisphere (left and right) entered as within-subject factors and group (total depressed versus controls) entered as the between-group factor. For the amygdala, this analysis was performed with the 9 substructure volumes and hemisphere (left and right) entered as within-subject factors and group (total depressed versus controls) entered as the between-group factor. Age, sex and eTIV were entered as covariates. Greenhouse-Geisser correction (Greenhouse and Geisser, 1959) was applied for all tests and any significant main effects or significant interactions were further examined using independent post-hoc univariate analysis of covariance (ANCOVA) with age, sex and eTIV as nuisance factors.

SECONDARY ANOVA (COMPOSITES)

A second mixed-model ANOVA was performed to investigate group-wise differences in volume of our defined hippocampal and amygdala composite measures. Hippocampal composite measures were the hippocampus proper (HP) hippocampal formation (HF) and hippocampal extended (HE). Amygdala composite measures were the laterobasal, centromedial and superficial groups of nuclei. In these analyses, either hippocampal or amygdala composite measures and hemisphere were entered as within-subject factors and group (total depressed versus controls) as the between-group factor. Age gender and eTIV were entered as covariates in the model. Again, any significant main effect and interactions were further investigated using post-hoc ANCOVA with age, sex and eTIV as nuisance factors.

TERTIARY ANOVA (FPD AND RD)

A third mixed-model ANOVA was performed to investigate group-wise differences in volumes of our defined hippocampal substructures and composites, with the depressed group divided into FPD and RC subgroups. Hippocampal substructures and

composites were entered as within-subject factors and group (FPD versus controls, or RD versus controls) as the between group factors. Age gender and eTIV were entered as covariates in the model. Again, any significant main effect and interactions were further investigated using post-hoc ANCOVA with age, sex and eTIV as nuisance factors. This was not done the amygdala measures due to time constraints.

POST-HOC ANCOVA

In order to clarify the driving effects identified by any main effect or significant interaction from the ANOVAs, additional post-hoc ANCOVAs were used to compare between-group differences for each hippocampal and amygdala substructure and hemisphere independently. Age, sex, and eTIV were entered as covariates. The same methodology was employed for the composite measures using a similar approach. Multiple Comparison Correction (MCC) was performed using False Discovery Rate (FDR) correction (Benjamini, 2010). FDR can be considered a more targeted form of MCC correction. Traditional Bonferroni correction "punishes" all input p-values equally, by dividing the p-value by the number of tests (Sedgwick, 2014), whereas Benjamini-Hochberg (as a way to control the FDR) "punishes" p-values accordingly to their ranking (Benjamini, 2010). In studies where there are only a few tests, either method can be used. However, in conditions where there are a large number of individual tests (e.g. in this study with 26 hippocampal substructures, 13 left and 13 right), a Bonferroni correction will produce false negatives i.e. it will discard true observations. As such FDR can be particularly good for exploratory studies like this.

HIERARCHICAL REGRESSION

Finally, hippocampal and amygdala volumes with significant between-group differences were examined using two forms of regression analyses. To assess the ability of substructures to predict group (depressed or controls), a form of binomial logistic regression was used. To assess the ability of substructures to predict disease duration (log transformed), a form of linear regression was used. Both regression tests were undertaken while controlling for age, sex and eTIV. Multiple comparisons

were controlled for during hierarchical regression using FDR (see above). Age, sex and eTIV were entered in step one of each model as control variables. Step two of each model entered each significant volume as the dependent variable with disease duration (linear) or group (depressed or controls) entered as the independent variable and again controlling for age, sex and eTIV.

2.9.3. FORNIX AND STRIA TERMINALIS STATISTICAL ANALYSIS

Both the fornix and the stria terminalis were analysed as whole complete tracts and divided into sections for analysis (along-tract analysis). The fornix was divided into 29 sections and the stria terminalis into 28 sections, due to the differences in length of the tracts (see Chapters 5 and 6 for further details).

TRACT AVERAGED STATISTICS

A series of repeated-measures ANOVA (analysis of variance) controlled for age, sex and eTIV (estimated total intracranial volume) was used with left and right hemisphere diffusion microscopic metrics (FA, MD, AD and RD) and macroscopic tract measures (length, volume and number of streamlines) entered as the within subject factors and group as the between-subject factor. Descriptives for group means, SEM (standard error of the mean) and 95% confidence intervals (CI) were obtained for each metric.

ALONG-TRACT STATISTICAL INVESTIGATIONS

All data was inspected for outliers and extreme values via the *Explore* tool within SPSS. Multivariate ANCOVA (analysis of covariance) for each diffusion metric (FA, MD, AD and RD) was used to investigate the variation of each metric over the length of the tract of interest. Between-group p-statistics for each metric were extracted for each section. Multiple comparison correction (MCC) was performed using False Discovery Rate (FDR) correction (Benjamini, 2010). To guard against the known negative effect of outlier and extreme outlier values on multivariate statistical tests, all outliers were 'winsorized' (Hastings et al., 1947), which essentially limits the outlier

points to the calculated upper and lower 95% CI boundaries. This also ensured additional statistical power, as missing data did not factor in the subsequent “cleaned” data. Age, sex and eTIV was controlled for throughout the analyses.

CORTISOL

The cortisol awakening response was measured by calculating the area under the curve with respect to increase from baseline, AUCi (Doolin et al., 2017, Fekedulegn et al., 2007) (see Figure 2.21. and Figure 4.5. for examples). Split-group partial correlations were performed to examine the relationship between amygdala nuclei volume measures and cortisol awakening response measures AUCi for each hemisphere.

CHAPTER 3

THE HIPPOCAMPUS:

MORE THAN THE SUM OF ITS PARTS?

"Indelible in the hippocampus is the laughter...."

Dr Blasey Ford,
US Senate Judiciary Committee, Sept 26th 2018

3.1. INTRODUCTION

Dysfunction in the hippocampus, a key hub within the limbic system, is proposed in the neuropathology of major depressive disorder (MDD) (Campbell and MacQueen, 2004) (see Chapter 1.3. for further commentary). Various meta-analyses examining magnetic resonance imaging (MRI) of the hippocampus solely (Videbech and Ravnkilde, 2004, McKinnon et al., 2009, Cole et al., 2011), or as part of a greater limbic system analysis (Koolschijn et al., 2009, Kempton et al., 2011, Arnone et al., 2012) have found volume reductions of 4-10% in depression. Voxel-based morphometry also demonstrates hippocampal grey matter volume loss in depression (Du et al., 2012, Zhao et al., 2014, Wise et al., 2016). Although changes have been found in first episode patients, most evidence indicates that volume reduction is associated with chronicity of depressive illness and multiple episodes (Sheline et al., 1999, McKinnon et al., 2009, Frodl et al., 2002). A minority of studies show no differences in depression (Rusch et al., 2001, Greenberg et al., 2008, Dotson et al., 2009, Kempton et al., 2011, Phillips et al., 2015). However, global hippocampal volumetric change needs to be interpreted with caution due to the multifaceted nature of the relationship between volumetric changes and MDD (MacQueen and Frodl, 2011, Sheline, 2011). Hippocampal neuroimaging studies are often conducted on heterogeneous groups of MDD sufferers with different grades of severity and subtypes of depression, inconsistent states of relapse/remission and treatment status - all of which may be reflected in different changes in the hippocampus.

Further variability may occur because the hippocampus consists of interrelated deep substructures that combine organizationally to form the traditional functional unit (Abrous et al., 2005, Andersen, 2007, O'Mara and Tsanov, 2015). Definitions of what constitutes the hippocampus often lack consensus among researchers (Engelhardt, 2016) and may compromise inter-study comparison. This has been discussed in detail in Chapter 1, but some studies are of particular relevance to this chapter. In a database of 423 hippocampal MRI studies, approximately 60 different anatomical guidelines were used to calculate hippocampal volume (Geuze et al., 2005). Classically the hippocampus proper consists of the allocortical cornu ammonis (CA)

subfields 1-4 (Andersen, 1975, Andersen, 2007) (Chapter 1.3.2.). This core hippocampal circuit operates within the hippocampal formation that also includes the subiculum inferiorly and the dentate gyrus medially (Schultz and Engelhardt, 2014). Broader definitions again may include anatomically adjacent areas such as the presubiculum and parasubiculum, functionally connected areas such as the entorhinal and parahippocampal cortices and associated accessory white matter areas such as the fimbriae and fornix (Andersen, 2007). Substructure changes may influence total hippocampal volume and sub-field variations may be obscured when the hippocampus is examined as a whole. Some specific substructural changes have been reported. Dentate gyrus and CA1-3 volume reductions have been found in non-medicated, but not in medicated, depressed patients (Huang et al., 2013). CA3 volume reduction has been associated with depression in Parkinson's disease (Gyorfi et al., 2017). In one study the number of episodes of depression was found to have an impact on dentate (Treadway et al., 2015) and subiculum volumes (Wisse et al., 2015). Reductions in left CA3, dentate and subiculum have also been found in a study focusing on depressed females (Han et al., 2016). A recent study, however, failed to find significant substructure changes in MDD compared to bipolar disorder or healthy controls (Cao et al., 2017). However, the same group also found that CA, dentate and subiculum volumes were predictive of therapeutic response from electroconvulsive therapy using a machine learning algorithm (Cao et al., 2018). As can be seen, substructural finding in depression have been inconsistent (see Chapter 1, Table 1.1. for a complete list of hippocampal substructure differences found in MDD).

Varying hippocampal definitions is a known problem in neuroimaging research (Geuze et al., 2005) with multiple differing definitions used in previous research. Variable hippocampal volumes may also pose difficulties when attempting to generalise preclinical hippocampal research to clinical populations. Basic hippocampal research often investigates hippocampal function at individual substructural and circuit-based/combined substructural level (Stepan et al., 2015). An approach examining individual substructures could assemble individual substructures into anatomico-functional, called henceforth, *composite regions* and

may help bridge the elusive gap between clinical and preclinical hippocampal research (MacQueen and Frodl, 2011, Samuels et al., 2015). This approach would help to identify specific hippocampal pathology associated with, and functional consequences of hippocampal neuroimaging changes in MDD.

Increasing MRI field strengths and recent advances in automated tissue segmentation protocols have enabled examination of hippocampal substructures with ever increasing precision (Fischl et al., 2002a, Lim et al., 2012, Wisse et al., 2015). Software advances such as **FreeSurfer 6.0** can exploit high-resolution multimodal T1 and T2 MRI data by using both *in-vivo* and *ex-vivo* atlas probabilistic segmentation to identify more sub-regions with improved accuracy (Iglesias et al., 2015). See Chapter 2.5 for a more details. Automated approaches have advantages over presumed 'gold standard' manual segmentation because of improved reliability and, if adapted into routine neuroimaging research should increase inter-study comparability of future studies (Pipitone et al., 2014).

In this study hippocampal MRI findings in depressed and non-depressed groups were evaluated using this latest technology. Patients with first presentation depression (FPD) and recurrent depression (RD) were investigated using automated hippocampal substructure segmentation of high definition T1 and T2-FLAIR weighted images. Substructure volumes were combined using a novel approach according to defined anatomical criteria based on the literature (known as composite measures). These were used to examine whether predicted reductions in overall hippocampal volume depended on specific anatomical hippocampal definitions. Exploratory composites were also created to further investigate the importance of particular sub-regions.

3.1.1. HYPOTHESES

My **hypotheses** were that

1. whole hippocampal volumes are smaller in MDD,
2. the more specific the hippocampal definition (i.e. the core CA regions), the greater the degree of difference between MDD and controls,
3. only the principal hippocampal substructures show differences in MDD,
4. chronic MDD shows smaller hippocampi than first presentation MDD, and
5. exploratory composites can provide further information about MDD than just the individual computed substructures.

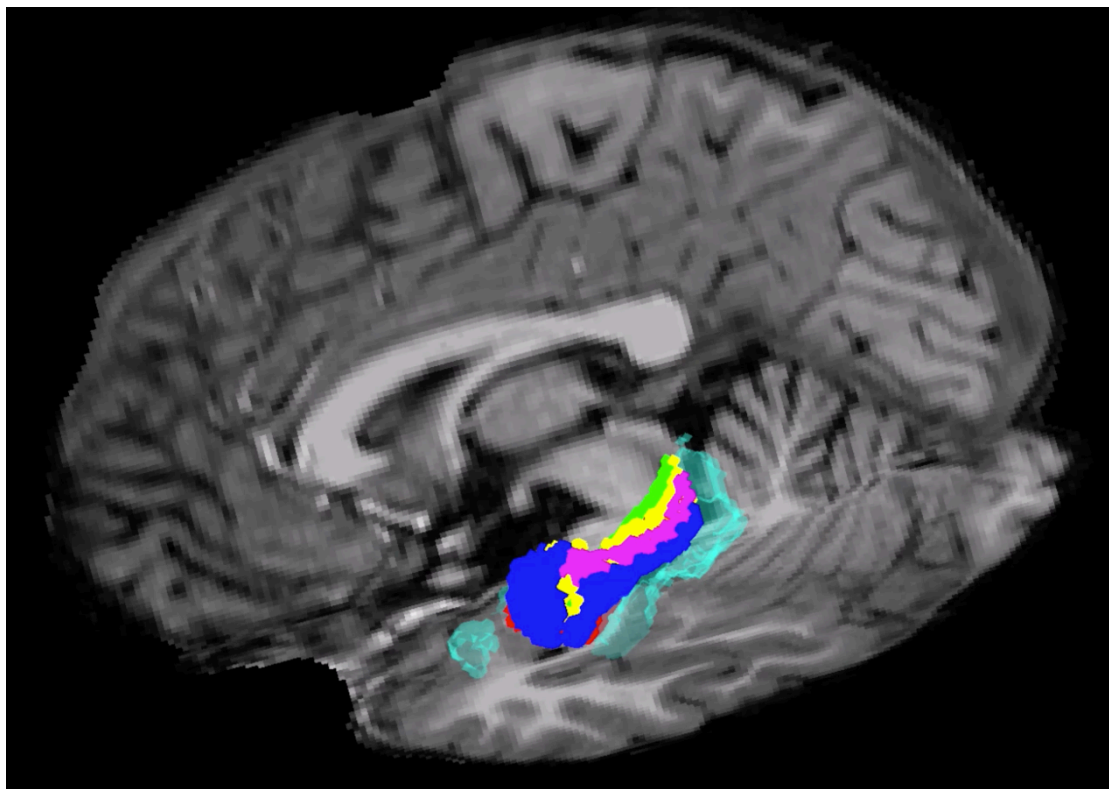


Figure 3.1. FreeSurfer 6.0 output showing hippocampus substructures. Axial and sagittal T1 image showing left hippocampus. Four substructures are shown. Blue; CA1; purple CA2-3, yellow, dentate gyrus; green, subiculum. The semi-transparent blue is the entorhinal cortex

3.2. METHODS

The methods used for this study are described in detail in Chapter 2.2., Chapter 2.3., Chapter 2.4. and Chapter 2.5. and Chapter 2.9. A brief summary description is given below.

Eighty people with Major Depressive Disorder (MDD) were compared to eighty-three healthy controls without MDD. All participants completed the MINI (Mini International Neuropsychiatric Interview) for DSM IV (American Psychiatric Association, 1994) and Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960). All patients required a current MINI diagnosis of MDD with a HAM-D greater than 20. Controls were required to have no active or previous MINI diagnosis and a HAM-D less than 8. Depressed patients were further subdivided into First Presentation Depression (FPD) and Recurrent Depression (RD) groups, based on the number of previous episodes of depression (FPD=1; RD $\geq$ 2).

3.2.1. MRI ACQUISITION

All data was acquired on a Philips (Best, Netherlands) Intera Achieva 3.0 Tesla MR system (32-channel head coil) at Trinity College Institute of Neuroscience, Dublin.

T1. 180 axial high-resolution T1-weighted anatomical images were acquired (T1W-IR1150 sequence, TE = 3.8 ms, TR = 8.4 ms, FOV 230 mm, 0.898 x 0.898 mm², in-plane resolution, slice thickness 0.9 mm, flip angle alpha = 8°).

T2-FLAIR. 60 axial T2-FLAIR images (TE = , TR = , 0.49 mm x 0.49 mm in-plane resolution, slice thickness 3 mm, flip angle alpha = 8°). Slices were taken in the axial plane, with the resulting smaller in plane resolution corresponding to the longitudinal axis of the hippocampus.

3.2.2. IMAGE ANALYSES

Cortical reconstruction and segmentation was performed using the *FreeSurfer* 6.0 image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>) (Fischl, 2012a, Fischl and Dale, 2000). The technical details of these procedures are described elsewhere (Fischl et al., 2002b, Desikan et al., 2006, Fischl et al., 1999, Fischl et al., 2004, Reuter et al., 2012, Dale et al., 1999). *FreeSurfer* interrogates T1 and T2 contrast differences between substructures using previously defined in-vivo and ex-vivo amygdala atlases to determine substructure characteristics. By combining T1 and T2-Flair inputs and selecting the 3T MRI flag and multispectral segmentation in *FreeSurfer* the procedure was optimized. 12 substructures were computed (Table 3.1.). See also Chapter 2, Table 2.3. for details of what anatomical landmarks *FreeSurfer* 6.0 uses to segment out individual substructures.

COMPOSITE MEASURES

Computed substructure volumes were summed together to generate novel composite measures. I initially generated the most restrictive hippocampal definition: the hippocampus proper (HP). This was calculated through summation of the core CA regions only. Secondly, a more functional hippocampal definition was calculated: the hippocampal formation (HF) comprising, in addition to the core CA regions, the dentate, subiculum and the tail (the tail consists largely of CA and dentate regions). This incorporated all the principal computed substructures involved in classic hippocampal circuitry. Thirdly a more expansive definition, hippocampal extended (HE), was defined incorporating all computed *FreeSurfer* 6.0 hippocampal substructures. Four exploratory anatomical regions (complete dentate, CA only, combined dentate /CA, and CA2-4 only regions) were also created (see Table 3.2. for complete details of how these composites were created) to further examine deeper hippocampal structure and interrelationships. See also Chapter 2, Table 2.5.

3.2.3. STATISTICAL ANALYSES

See Chapter 2.9. for further details. All extracted subfield volumes were systematically inspected visually, and measures exported to SPSS24 (<https://www.ibm.com/analytics/us/en/technology/spss/>). Outlier volumes were removed manually to ensure that all data fell within the 95% confidence interval. This systematic removal of outliers ensured that extreme or atypical data points did not unduly influence the following analysis. The depressed group was analysed initially as a complete group and then subsequently as distinct FPD and RD sub-groups.

A primary mixed-method-analysis of variance (ANOVA) was used to investigate group-wise differences in substructure volumes across all substructures and hemisphere (left and right) of the hippocampi. This analysis was performed with the 12 substructure volumes and hemisphere (left and right) entered as within-subject factors and group (total depressed versus controls) entered as the between-group factor. Age, sex and estimated total intracranial volume (eTIV) were entered as covariates. Greenhouse-Geisser correction (Greenhouse and Geisser, 1959) was applied for all tests and any significant main effects or significant interactions were further examined using independent post-hoc univariate analyses of covariance (ANCOVA) with age, sex and eTIV as nuisance factors. Partial eta² describes effect size (0.01=low, 0.06=moderate, 0.14=large) (Cohen, 1988).

A second mixed-method-ANOVA was performed to investigate group-wise differences in volume of our defined whole hippocampal composite measures (i.e. HP, HF and HE). In this analysis, composite measures and hemisphere were entered as within-subject factors and group (total depressed versus controls) as the between-group factor. Age gender and eTIV were entered as covariates in the model. Again, any significant main effect and interactions were further investigated using post-hoc ANCOVA with age, sex and eTIV as nuisance factors.

Finally, an additional mixed-method-ANOVA was performed to investigate group-wise differences in volume with the depressed cohort subdivided into RD and FPD subgroups compared to controls for our defined composite measures (HP, HF, and HE) of the hippocampus. In this analysis, composite measures and hemisphere were entered as within-subject factors and group (RD, FPD (depressed) versus controls) as the between-group factor. Age, gender and eTIV were entered as covariates in the model. Again, any significant main effect and interactions were further investigated using post-hoc ANCOVA with age, sex and eTIV as nuisance factors with group further stratified as controls, FPD and RD.

In order to clarify the driving effects identified by any main effect or significant interaction from the ANOVAs, additional post-hoc ANCOVA were used to compare between-group differences for each substructure and hemisphere independently. Age, sex, and eTIV were entered as covariates. The same methodology was employed for the composite measures using a similar approach. Multiple Comparison Correction (MCC) was performed using False Discovery Rate (FDR) correction (Benjamini, 2010) .

HIERARCHICAL REGRESSION

Finally, volumes with significant between-group differences were examined using a two-step hierarchical multiple regression analyses to assess the ability of disease duration (log transformed) to predict substructure volumes while controlling for age, sex and eTIV. Multiple comparisons were controlled for during hierarchical regression using the Bonferroni correction applied at $p=0.05$. Age, sex and eTIV were entered in step one of the model as control variables. Step two of the model entered each volume as the dependent variable with disease duration entered as the independent variable and again controlling for age, sex and eTIV.

Substructure
CA1
CA2/3
CA4
Dentate
Subiculum
Presubiculum
Parasubiculum
Tail
Molecular layer
HATA
Fimbriae
Fissure

Table 3.1. Computed hippocampal substructures. 12 substructures were generated. See also Figure 3.2. for a schematic of the computed substructures. See also Chapter 2, Table 2.3. for a complete description of the anatomical landmarks *FreeSurfer* 6.0 uses to calculate the substructures. CA, cornu ammonis; HATA, hippocampal amygdala transition area.

Composite Measures	Substructure volumes added
Hippocampal Extended	CA1, CA2/3, CA4, Dentate, Subiculum, Tail, Molecular Layer, Presubiculum, Parasubiculum, HATA, Fimbriae.
Hippocampal Formation	CA1, CA2/3, CA4, Dentate, Subiculum, Tail
Hippocampal Proper	CA1, CA2/3, CA4
Combined Dentate	CA4, Dentate
CA Only	CA1, CA2/3
Combined Dentate/CA	Dentate, CA1, CA2/3, CA4
CA2-4	CA2/3, CA4

Table 3.2. Hippocampal composites. Schematic hippocampal diagrams with the composite measures accentuated can be found in Figure 3.3., Figure 3.4., Figure 3.5., and Figure 3.6. Further explanations and definitions can be found in Chapter 2, Table 2.5. . CA, cornu ammonis; HATA, hippocampal amygdala transition area.

3.3. RESULTS

No significant between-group differences between controls and total depressed or between controls and FPD were found for age, sex or handedness. However, the RD group was different from both the controls and FPD group, with the RD having a higher proportion of females as well as being significantly older (10-12 years older). While HAM-D was higher in patients compared to controls, no difference was found between FPD and RD groups. The HAM-D for the FPD group was 21, considered moderate depression, whereas the HAM-D for the RD group was 23, considered severe depression. The average duration of depression in FPD and RD was 10 months and 60 months respectively. All RD patients and 73% of FPD patients (27/37) were taking antidepressants at the time of scanning.

	Group				Con v Dep	Con v FPD	Con v RD	FPD v RD
	Con N=83	Dep N=80	FPD N=43	RD N=37	<i>p</i> -value			
Age: Mean(SEM)	31.5(1.4)	34.5(1.4)	29(1.7)	41(1.8)	0.13	0.22	0.0001	3.90E-06
Range (years)	16-64	17-64	17-61	22-64	-----	-----	-----	-----
Male/Female (%M)	34/49, 41%	23/57, 29%	19/24, 44%	4/33, 11%	X ² : 0.14	X ² : 0.85	X ² : 0.001	X ² : 0.001
Handedness (R/L)	75/8	73/7	-----	-----	NS	-----	-----	-----
HAMD* (SEM)	1.3(0.4)	22.2(0.4)	21.4(0.4)	23(0.6)	1.10E-75	1.98E-65	2.00E-53	0.04
MDD months**	-----	29(4)	10(4)	60(5)	-----	-----	-----	-----

Table 3.3. Demographic Data. Con, controls; Dep, depressed; HAM-D, Hamilton Depression scale; M, male; MDD, major depressive disorder; R/L, right or left handed; SEM, standard error of the mean.

3.3.1. HIPPOCAMPAL VOLUMETRICS

The primary mixed-method ANOVA revealed a strong trend towards significance for the main effect for group (Controls versus *all* depressed), ($F = 3.69$ (1dof), $p=0.057$, partial $\eta^2 = 0.03$, power = 0.48). The first within-subject factor (hemisphere) failed to identify a significant main effect for hemisphere when collapsed across groups indicating that, overall, left and right hippocampal volumes were similar. However, a significant group x hemisphere interaction ($F = 6.55$ (1 dof), $p=0.012$, partial $\eta^2 = 0.05$, power=0.72) was found which indicated that although there was no difference in hemisphere, group significantly influenced this with the depressed group demonstrating a consistent bilateral pattern of reduced subfield volumes compared to controls with the left hemisphere being more impacted and having smaller

volumes (see Table 3.4. and Table 3.5.). The second within-subject factor (substructure) identified a significant main effect for substructure volume ($F = 6.91$ (3.2dof), $p=0.0001$, partial $\eta^2 = 0.05$, power = 0.98), which indicated that the 12 subfield volumes differed, with a diverse pattern of subfield specific anatomical variance within the hippocampus. Group x hemisphere x substructure also identified a significant interaction ($F = 6.89$ (2.68 dof), $p=0.0004$, partial $\eta^2 = 0.05$, power=0.96) that revealed a complex relationship between substructure volume and hemisphere that differed between controls and depressed.

Post hoc ANCOVA tests revealed that this interaction was driven by smaller volumes bilaterally for CA3, CA4, subiculum and dentate in depressed and lateralized differences with smaller tail and CA1 volumes on the left and larger right ML volumes for the depressed group. Overall, a more extensive pattern of smaller substructure volumes in the depressed group was evident for the left hippocampus. Similar patterns were seen for the right hemisphere but to a lesser extent. Lateralized effects specific to the CA1 and molecular layer were also identified.

ANCOVA with subsequent MCC for each substructure revealed significant bilateral volume reductions in depression in four substructures: the subiculum, dentate, CA2/3 and CA4 (See 3.4. and Table 3.5.). The CA1 region and the hippocampal tail were reduced on the left side only. The molecular layer, in contrast to all other structures, showed a right-sided increase in depression. In the FPD group, CA2/3 and CA4 were reduced on the left with no significant substructure changes on the right. Partial correlation analyses failed to identify significant correlation for duration of depression and associated substructure volumes when all patients with depression were combined.

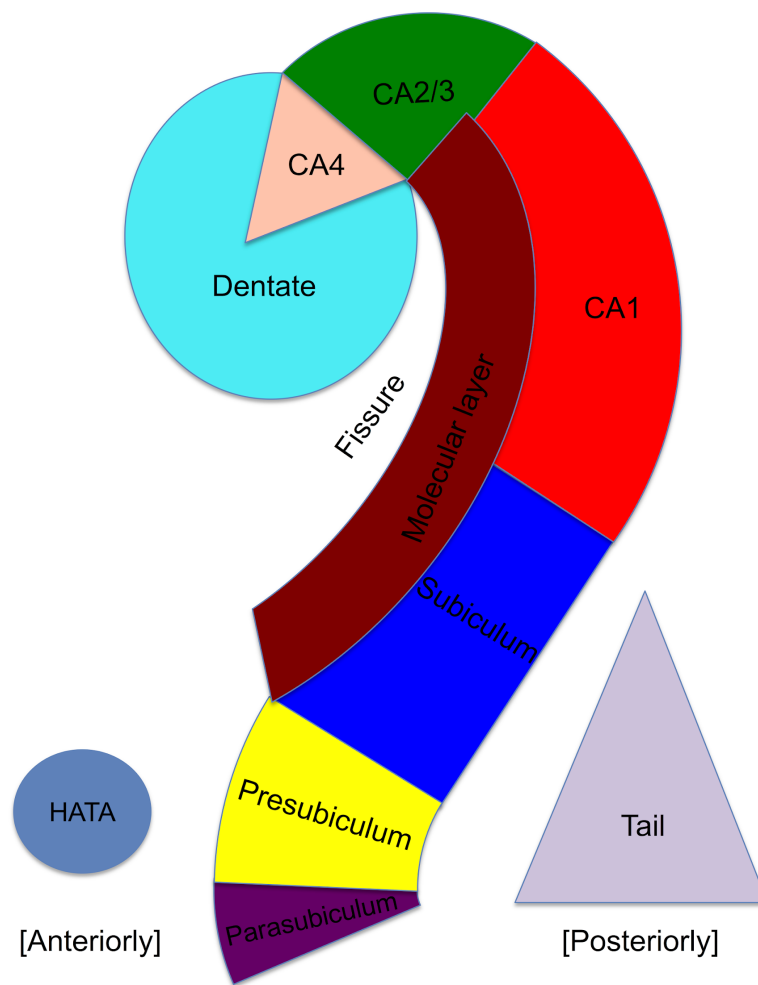


Figure 3.2. Hippocampal substructures. Schematic cross-sectional view of hippocampal substructures based on *FreeSurfer* 6.0 output. This is used as the basis for further cross-sectional schematics used to illustrate the whole hippocampal volume changes (Figure 3.3.), first presentation versus recurrent depression changes (Figure 3.5.) and exploratory composite changes (Figure 3.6.). The HATA and tail are not cross-sectional but placed either side for ease of viewing. CA, cornu ammonis; HATA, hippocampal amygdala transition area.

Substructure	Left						
	Con	Dep	Con vs Dep	Effect Size	Con vs FPD	Con vs RD	Effect Size
	Mean (SEM) mm ³		p-value	(partial eta ²)	p-value		(partial eta ²)
CA1	663(7)	627(7)	0.0004 ↓ (78/77)	0.08	0.021↓ (78/42)	0.006 ↓ (78/35)	0.08
CA2/3	239(3)	223 (3)	0.0001 ↓ (76/77)	0.09	0.002 ↓ (76/41)	0.018 ↓ (76/36)	0.09
CA4	265(3)	252(3)	0.001 ↓ (80/77)	0.07	0.0063 ↓ (80/41)	0.005 ↓ (80/36)	0.08
Dentate	319(3)	305(3)	0.002 ↓ (79/77)	0.06	0.120↓ (80/41)	0.008 ↓ (80/36)	0.07
Subiculum	413(5)	392(5)	0.001 ↓ (78/75)	0.06	0.094↓ (78/40)	0.004 ↓ (78/35)	0.08
Tail	544(6)	522(6)	0.016 ↓ (81/75)	0.04	0.999↓ (81/41)	0.003 ↓ (81/34)	0.07
Molecular Layer	485(7)	499(7)	0.181↑ (79/76)	0.01	0.615↑ (79/42)	0.999↑ (79/34)	0.01
Presubiculum	267(3)	270(3)	0.501↑ (81/77)	0.003	0.999↑ (81/41)	0.999↑ (81/36)	0.003
Parasubiculum	62(1)	63(1)	0.632↑ (80/77)	0.002	0.999↑ (80/42)	0.999↑ (80/36)	0.002
Fimbria	82(2)	81(2)	0.837↑ (80/76)	0.0005	0.999↑ (80/40)	0.999↑ (80/36)	0.001
Fissure	115(2)	119(2)	0.175↑ (79/75)	0.012	0.391↑ (79/39)	0.999↑ (79/36)	0.016
HATA	68(1)	68(1)	0.905↑ (81/76)	0.0005	0.999↑ (81/41)	0.999↑ (81/36)	0.0005
FDR across all measures			0.021		0.006	0.025	

Table 3.4. Left hippocampal between group differences for individual substructures following ANCOVA correcting for age, sex and eTIV and also after correcting for multiple comparisons using FDR. Representative volumes are shown in mm³ with standard deviations in the first two data columns. Numbers in parenthesis in p-value columns (column 3) show actual numbers following removal of outliers ensuring all data fell within the 95% confidence interval. Partial eta² describes effect size (0.01=low, 0.06=moderate, 0.14=large). Bold text indicates p-values that survived FDR correction or moderate to large effect size. FDR for each group is found at the bottom of the table. ANCOVA, analysis of covariance; CA, cornu ammonis; Con, controls, Dep, depressed; eTIV, estimated total intracranial volume; FDR, false discovery rate; FPD, first-presentation depression; HATA, hippocampal amygdala transition area; RD, recurrent depression.

Substructure	Right						
	Con	Dep	Con vs Dep	Effect Size	Con vs FPD	Con vs RD	Effect Size
	Mean (SEM) mm ³		p-value	(partial eta ²)	p-value		(partial eta ²)
CA1	649(7)	634(7)	0.120 (79/77)	0.016	0.456↓ (79/42)	0.951↓ (79/35)	0.02
CA2/3	248(3)	236(3)	0.003 ↓ (80/77)	0.06	0.014↓ (80/42)	0.179↓ (81/35)	0.06
CA4	270(3)	258(3)	0.003 ↓ (78/77)	0.06	0.149↓ (78/42)	0.014↓ (79/35)	0.06
Dentate	325(3)	311(3)	0.004 ↓ (79/77)	0.05	0.166↓ (79/42)	0.018↓ (79/35)	0.06
Subiculum	400(4)	383(4)	0.006 ↓ (79/76)	0.05	0.265↓ (79/41)	0.014↓ (79/35)	0.06
Tail	539 (7)	527(7)	0.267↓ (81/78)	0.008	0.883↓ (81/42)	0.883↓ (80/36)	0.008
Molecular Layer	505(8)	537(8)	0.006 ↑ (81/78)	0.05	0.120↑ (81/42)	0.056↑ (81/36)	0.05
Presubiculum	256(3)	264(3)	0.066↑ (81/78)	0.02	0.272↑ (81/42)	0.706↑ (81/36)	0.02
Parasubiculum	59(1)	62(1)	0.064↑ (81/78)	0.03	0.734↑ (81/42)	0.184↑ (81/36)	0.03
Fimbria	81(2)	78(2)	0.344↓ (80/77)	0.006	0.659↓ (81/42)	0.999↓ (81/36)	0.01
Fissure	117(2)	122(2)	0.139↑ (81/78)	0.014	0.999↑ (81/42)	0.366↑ (81/36)	0.02
HATA	66(1)	67(1)	0.384↑ (81/78)	0.005	0.999↑ (81/42)	0.730↑ (81/36)	0.009
FDR across all measures			0.013		0	0.009	

Table 3.5. Right hippocampal between group differences for individual substructures following ANCOVA correcting for age, sex and eTIV and also after correcting for multiple comparisons using FDR. Representative volumes are shown in mm³ with standard deviations. Numbers in parenthesis show actual numbers following removal of outliers ensuring all data fell within the 95% confidence interval. Partial eta² describes effect size (0.01=low, 0.06=moderate, 0.14=large). Bold text indicates p-values that survived FDR correction or moderate to large effect size. FDR for each group is found at the bottom of the table. ANCOVA, analysis of covariance; CA, cornu ammonis; Con, controls, Dep, depressed; eTIV, estimated total intracranial volume; FDR, false discovery rate; FPD, first-presentation depression; HATA, hippocampal amygdala transition area; RD, recurrent depression; SEM, standard error of the mean.

3.3.2. COMPOSITE MEASURES

The initial mixed-model ANOVA for composite measures revealed a significant main effect for group; Greenhouse-Geisser ($F = 6.97$ (1dof), $p=0.009$, partial $\eta^2 = 0.045$, observed power $= 0.75$). The first within-subject factor (hemisphere, left and right) failed to identify a significant main effect for hemisphere when collapsed across groups indicating that overall, left and right hippocampal volumes were similar. However, a significant group x hemisphere interaction; Greenhouse-Geisser ($F = 7.66$ (1 dof), $p=0.006$, partial $\eta^2 = 0.05$, power $= 0.79$) was found indicating that although there was no difference in hemisphere, group significantly influenced this with the depressed group demonstrating a consistent bilateral pattern of reduced composite volumes compared to controls with the left hemisphere being more impacted and having smaller composite volume measures.

The second within-subject factor (composite measures, HP, HF and HE) identified a significant main effect for composite volume; Greenhouse-Geisser ($F = 20.94$ (1.1dof), $p=5.0E-6$, partial $\eta^2 = 0.13$, power $= 0.99$), which indicated that the three composite volumes significantly differed. Hemisphere x composite interaction was not significant showing that the composite measures were not largely different between the left and right hemispheres when collapsed across depressed and control groups. The driving effect behind this main effect was further clarified when group x composite x hemisphere interactions were examined; Greenhouse-Geisser ($F = 4.99$ (dof1.18), $p=0.022$, partial $\eta^2 = 0.033$, observed power $= 0.65$). Group differences had a clear effect in this complex relationship with depressed composite measures revealing a consistent pattern of smaller bilateral composite volumes relative to controls. A further group specific pattern was also evident with right composite volumes being larger than left for the depressed group, a pattern that was not present in controls.

Finally, a further mixed-method ANOVA with age, gender and eTIV as covariates but with a further stratification of the depressed group into the two RD and FPD subgroups compared to controls revealed an interesting pattern. Smaller composite volumes were found in the RD group compared to the FPD and lateralized to the left hemisphere. Similar results to the previous all depressed vs controls composite analysis were seen: significant main effect for group ($F = 6.97$ (1dof), $p=0.024$, partial $\eta^2 = 0.05$, observed power = 0.69), significant group x hemisphere interaction ($F = 5.09$ (2 dof), $p=0.007$, partial $\eta^2 = 0.065$, power = 0.81), and significant main effect for composite volume ($F = 20.96$ (1.1dof), $p=5.0E-6$, partial $\eta^2 = 0.13$, power = 0.99). In all ANCOVA analyses, Levene's Test of Equality of Error Variances was checked, and no violations were found (Levene, 1961).

All total hippocampal composite volumes showed reductions on the left in depression (Table 3.6. and Table 3.7.) relative to controls. Only the core HP showed a volume change on the right. As the three composites of total hippocampal volume became progressively more focused towards core hippocampal substructures rather than expansive definitions (i.e. HE → HF → HP), the significance of the change in depression compared to controls increased in a sequential fashion on both the left ($p=0.0152 \rightarrow p=0.0013 \rightarrow p=0.0002$) and right ($p=0.2118 \rightarrow p=0.114 \rightarrow 0.129$). See 3.3. and Figure 3.4.

	Left						
	Con	Dep	Con vs Dep	Effect Size	Con vs FPD	Con vs RD	Effect Size
	Mean (SEM) mm ³		p-value	(partial eta ²)	p-value		(partial eta ²)
Hippocampal Composites							
Hippocampal Extended	3442(279)	3291(334)	0.005 ↓ (80/76)	0.05	0.261↓ (80/42)	0.009 ↓ (80/34)	0.07
Hippocampal Formation	2938(22)	2833(23)	0.003 ↓ (80/76)	0.05	0.118↓ (80/42)	0.021 ↓ (80/35)	0.06
Hippocampal Proper	1168(12)	1104(12)	0.0002 ↓ (79/77)	0.09	0.0041 ↓ (79/42)	0.0049 ↓ (79/35)	0.09
Anatomical Composites							
Complete Dentate	587(6)	558(6)	0.0004 ↓ (79/77)	0.06	0.0087 ↓ (79/41)	0.0028 ↓ (79/36)	0.06
CA Only	899(9)	849(9)	0.0003 ↓ (78/77)	0.09	0.0058 ↓ (77/42)	0.0051 ↓ (79/35)	0.09
Combined Dentate/CA	1487(15)	1403(15)	0.0001 ↓ (79/76)	0.08	0.0021 ↓ (79/41)	0.0042 ↓ (79/35)	0.08
CA2-4	505(5)	477(6)	0.0002 ↓ (78/77)	0.07	0.0013 ↓ (78/41)	0.0080 ↓ (78/35)	0.07
FDR across all measures			0.021		0.006	0.025	

Table 3.6. Left hippocampal between group differences for all composite measures following ANCOVA correcting for age, sex and eTIV and also after correcting for multiple comparisons using FDR. Total hippocampal volume and anatomical composite volume differences are shown. Representative volumes are shown in mm³ with standard deviations. Numbers in parenthesis show actual numbers following removal of outliers ensuring all data fell within the 95% confidence interval. Partial eta² describes effect size (0.01=low, 0.06=moderate, 0.14=large). Bold text indicates p-values that survived FDR correction or moderate to large effect size. ANCOVA, analysis of covariance; CA, cornu ammonis; Con, controls; dep, total depression; eTIV, estimated total intracranial volume; FDR, false discovery rate; FPD, first presentation depression; HATA, hippocampal amygdalar transition area; MDD, major depressive disorder; RD, recurrent depression; SEM, standard error of the mean.

	Right						
	Con	Dep	Con vs Dep	Effect Size	Con vs FPD	Con vs RD	Effect Size
	Mean (SEM) mm ³		p-value	(partial eta ²)	p-value		(partial eta ²)
Hippocampal Composites							
Hippocampal Extended	3398(25)	3353(25)	0.232↓ (80/75)	0.01	0.3498↓ (80/41)	0.4208↓ (80/34)	0.01
Hippocampal Formation	2931(21)	2881(22)	0.121↓ (79/76)	0.02	0.2493↓ (79/41)	0.2757↓ (79/34)	0.02
Hippocampal Proper	1168(11)	1128(11)	0.0129 ↓ (79/77)	0.04	0.3742↓ (80/41)	0.8751↓ (80/34)	0.05
Anatomical Composites							
Complete Dentate	598(6)	568(6)	0.003 ↓ (78/75)	0.06	0.080↓ (78/40)	0.030↓ (78/35)	0.06
CA Only	897(9)	870(9)	0.034↓ (79/77)	0.03	0.078↓ (79/42)	0.1816↓ (79/35)	0.03
Combined Dentate/CA	1498(14)	1442(14)	0.010 ↓ (80/77)	0.04	0.061↓ (80/42)	0.0572↓ (80/35)	0.04
CA2-4	521(5)	495(6)	0.002 ↓ (81/77)	0.06	0.022↓ (81/42)	0.108↓ (81/35)	0.06
FDR across all measures			0.013		0	0.009	

Table 3.7. Right hippocampal between group differences for all composite measures following ANCOVA correcting for age, sex and eTIV and also after correcting for multiple comparisons using FDR. Total hippocampal volume and anatomical composite volume differences are shown. Representative volumes are shown in mm³ with standard deviations. Numbers in parenthesis show actual numbers following removal of outliers ensuring all data fell within the 95% confidence interval. Partial eta² describes effect size (0.01=low, 0.06=moderate, 0.14=large). Bold text indicates p-values that survived FDR correction or moderate to large effect size. ANCOVA, analysis of covariance; CA, cornu ammonis; Con, controls; dep, total depression; eTIV, estimated total intracranial volume; FDR, false discovery rate; FPD, first presentation depression; HATA, hippocampal amygdalar transition area; MDD, major depressive disorder; RD, recurrent depression; SEM, standard error of the mean.

Finally, exploratory analysis of anatomical composites (Complete Dentate, CA-only, Combined Dentate/CA and C2-4) showed volume reductions across all composites on the left in depression, driven by both FPD and RD groups. No differences were seen on the right. The left side showed reduced volumes of the complete dentate, CA only, combined dentate/CA, and C2-4 regions, with the complete dentate being driven by RD (Table 3.6. and Table 3.7.).

All the above results are reported as significant only after applying strict correction for multiple comparisons, using the False Discovery Rate (Benjamini, 2010). Trend level, non-significant results can be seen in Table 3.4., Table 3.5., Table 3.6. and Table 3.7.

3.3.3. HIERARCHICAL REGRESSION

Hierarchical multiple regression revealed that the left CA1 volume emerged as the only substructure with a predictive relationship between disease duration and substructure volume. No other structure showed a predictive relationship on either side. This step accounted for 16% of the variance in CA1. Step two of the model showed that the total variance explained in CA1 was 22% $F(4,63) = 4.31, p < 0.005$. An additional 6% of the variance in CA1 was explained by disease duration, R^2 change = 0.057, F change $(1,63) = 4.6, p = 0.036$ correcting for age, sex and eTIV. The final model identified two significant measures, eTIV ($\beta = 0.29, p = 0.025$), and duration of depression ($\beta = 0.28, p = 0.036$), although the effect of eTIV was subsequently controlled for and hence removed.

3.4. DISCUSSION

This study investigated hippocampal substructural volumes in eighty depressed individuals and eighty-three healthy controls using advanced automated segmentation. Extensive bilateral volume reductions across multiple principal substructures were found in MDD, with left core hippocampal reduction being the most salient finding. Differences were generally more pronounced on the left and with increasing chronicity of depression. Analysing the hippocampus using novel composite anatomical definitions highlighted the importance of core CA regions over more peripheral areas in the pathogenesis of depression. First presentation depression showed CA2-4 changes only while patients with recurrent depression had more extensive pattern of substructure involvement. It is likely that this reflects potential evolving hippocampal deterioration associated with increasing chronicity of depression.

3.4.1. HYPOTHESIS 1: WHOLE HIPPOCAMPAL VOLUMES ARE SMALLER IN MDD

Extensive volume differences were found between MDD and control groups, with total hippocampal volumes showing reductions when compared to controls. Findings for total hippocampal volumes in depression are consistent with many studies showing hippocampal volume reductions in first episode (Frodl et al., 2002, Cole et al., 2011) and chronic patients (Videbech and Ravnkilde, 2004, McKinnon et al., 2009). Even though hippocampal volumes global showed reductions in MDD, the degree of difference depended on the exact hippocampal definition (see hypothesis 2 , Chapter 3.4.2. below).

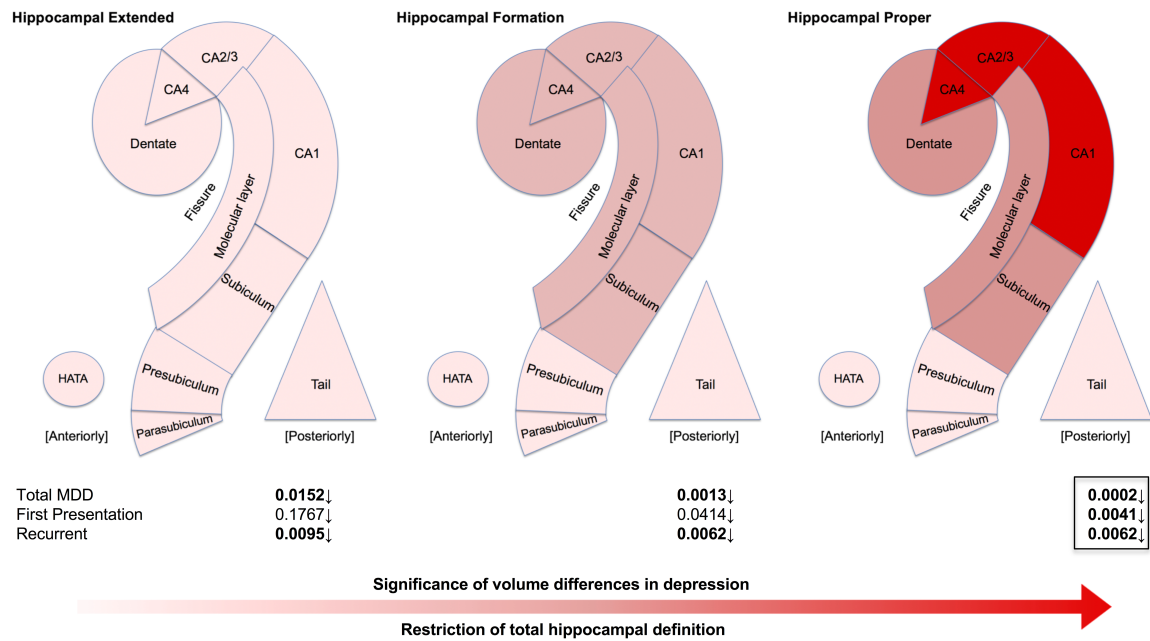


Figure 3.3. Total hippocampus volumes in depression 1. Schematic cross-sections of the three different left total hippocampal definitions. Hippocampal Extended includes all computed substructures. As the hippocampal volume is restricted, the significance of the differences in total MDD, FPD and RD increases. See Table 3.2. and Figure 3.4. CA, cornu ammonis; FPD, first presentation depression; HATA, hippocampal amygdalar transition area; MDD, major depressive disorder; RD, recurrent depression.

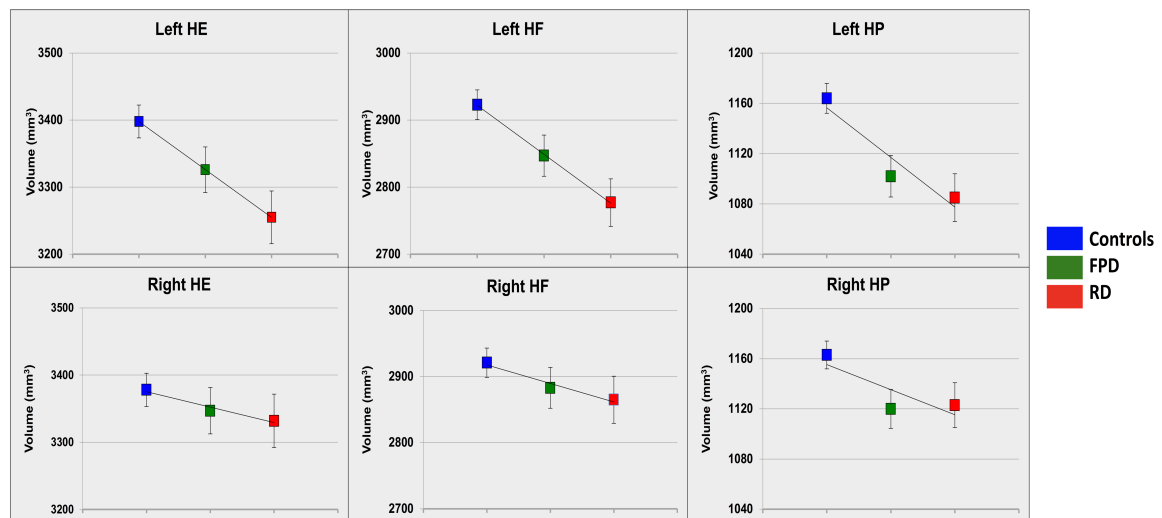


Figure 3.4. Total hippocampus volumes in depression 2. Left and right hippocampal volumes in controls, FPD and RD. HE includes all computed substructures. HF includes only dentate, subiculum and CA substructures. HP includes CA substructures only. See Table 3.2. for further details on composite assembly. See Figure 3.3. for a schematic representation of changes of volume between controls and depressed. See Table 3.6. and Table 3.7. for composite values. Note the overall downward trend across hippocampal definitions for total hippocampal volumes with chronicity. CA, cornu ammonis; FPD, first presentation depression; HE, hippocampal extended; HF, hippocampal formation; HP, hippocampal proper; RD, recurrent depression.

3.4.2. HYPOTHESIS 2: THE MORE SPECIFIC THE HIPPOCAMPAL DEFINITION (I.E. THE CORE CA REGIONS), THE GREATER THE DEGREE OF DIFFERENCE BETWEEN MDD AND CONTROLS

There are recent moves towards developing a harmonized whole hippocampus segmentation protocol (Bocchetta et al., 2015, Boccardi et al., 2015). This is first published study, at the time of writing the thesis, in which multiple composite definitions of the hippocampus were used to investigate hippocampal volumes in depression. Three total hippocampal composite volumes were calculated through the summation of appropriate substructures (Figure 3.3 and Figure 3.4). Using the broadest definitions of hippocampal volume – hippocampal formation (HF) and hippocampus extended (HE) – showed a reduced hippocampus in depression exclusively on the left side which was attributable to differences in the RD group. The most restrictive definition of hippocampus, hippocampus proper (HP) yielded volume differences bilaterally in MDD, and, unlike the HE and HF these volume reductions were maintained across first presentation and recurrent groups. Of note, the left core HP volume reduction in depression was highly significant ($p=0.0002$).

Using a novel approach, I built multiple 'ground-up' composite definitions of total hippocampal volumes by assembling substructural components according to the neuroanatomical literature. As the hippocampal definition became progressively constricted, from the larger non-specific HE through the more mid-sized functional HF and finally to the smaller highly specific HP, the volume reductions seen in depression became progressively more pronounced (Table 3.6. & Table 3.7., Figure 3.4.). The implications of this are twofold. Firstly, it highlights the importance of rigorous hippocampal definitions in studies of MDD. This indicates that differences present in the literature may be caused by differing hippocampal definitions. Previous non-significant findings examining a larger hippocampal corpus may obscure hippocampal substructural changes in depression (Dotson et al., 2009, Greenberg et al., 2008, Phillips et al., 2015, Rusch et al., 2001). A composite-based approach such as that described here may help resolve some inter-study

inconsistencies moving forward. Hippocampal definition is therefore important. Secondly, the conservative HP measure supports Hypothesis 2 that the *classic core* CA subfields are thoroughly involved in the MDD disease process. This means, in essence, **the more focused the hippocampal definition the greater the degree of change in depression**. Disruption of the CA regions could interfere with all aspects of the trisynaptic hippocampal memory circuit and alter fundamental sensory, memory and emotional information processing. The trisynaptic circuit allows entorhinal (EC) inputs to be processed through the key hippocampal subfields (EC → Dentate/CA4 → CA3 → CA1) before outputting through the subiculum (Stepan et al., 2015, Andersen, 2007). Disruption of any of these principal hippocampal substructures could result in neuropsychological difficulties common in symptoms (Samuels et al., 2015).

3.4.3. HYPOTHESIS 3: ONLY THE PRINCIPAL HIPPOCAMPAL SUBSTRUCTURES SHOW DIFFERENCES IN MDD

Substructure changes were found only in those considered part of the classic hippocampal formation i.e. CA1-4, dentate, subiculum, tail (composed largely of CA and dentate) and the molecular layer (the upper layer of the subiculum and CA). No differences were found for the *non-classic* hippocampal substructures of the presubiculum, parasubiculum, fimbria, HATA and the hippocampal fissure. Replicating findings of a recent substructural study, also using *FreeSurfer 6.0*, no correlation was found between MDD duration and any individual substructure volume (Cao et al., 2017). Differences in MDD were generally observed bilaterally in principal substructures with differences consistently more pronounced on the left and frequently more prominent in the recurrent subgroup. There were smaller differences on the right in MDD compared to the controls but the CA1 was showed no change on the right. Once again, my findings were broadly consistent with recent studies looking at hippocampal substructures in depressed patients (Huang et al.,

2013, Gyorfi et al., 2017, Han et al., 2016, Treadway et al., 2015). A novel finding in this present study is that the CA1 and hippocampal tail demonstrated lateralization of pathology exclusively to the left side. The tail comprises elements of all principal substructures (CA, dentate and subiculum). However, the relative dominance of CA1 within the hippocampal tail compared to other substructures may account for some of the lateralization found in both the CA1 region and the tail.

Molecular layer

Only one substructure showed a volume increase in depression. The right molecular layer measure was larger in MDD and driven by FPD only. This slightly unusual measure incorporates the upper cortical layer across the combined subiculum and CA1-3 regions (Iglesias et al., 2015) (see Chapter 1.3.2 for further details on the composition and unique segmentation of the molecular layer). Lining the fissure just below the cerebrospinal fluid, this layer is populated sparsely with neuronal bodies and contains connecting axons, interneurons and glial cells (Capogna, 2011). It is noteworthy that this was the only hippocampal substructure increased in depression. Potential explanations include an initial inflammatory response, glial scarring along the fissure edge or extensive dendritic branching in this layer in the early stages of the disease.

3.4.4. HYPOTHESIS 4: CHRONIC MDD SHOWS SMALLER HIPPOCAMPI THAN FIRST PRESENTATION MDD

The left CA1 exhibited reduced volumes across both first presentation and recurrent groups, but only differences in the recurrent group survived the strict FDR correction applied throughout the analysis. The CA1 region is particularly vulnerable to various insults (Zola-Morgan et al., 1986, Bartsch et al., 2015) and has been found in post-mortem studies of MDD patients to contain greater evidence of neuronal apoptosis relative to non-depressed controls (Lucassen et al., 2001). CA1 is involved in autobiographical memory (Bartsch et al., 2011), contextual memory retrieval

(Dimsdale-Zucker et al., 2018) and self-awareness (Danjo et al., 2018). MDD is also associated with autobiographical memory difficulties and overgeneralization deficits (Kohler et al., 2015). Left CA1 volume was the only individual component to survive regression analysis suggesting that left CA1 volume uniquely represents a predictor of MDD illness duration. CA1 tissue also has a particularly high expression of many 5-HT receptor subtypes (Pazos et al., 1987, Karten et al., 1999, Dale et al., 2016) and may be especially sensitive to disturbances of the serotonergic system or, alternatively, limbic 5-HT neurotransmission could be disrupted secondary to CA1 disease. Long-term corticosteroid exposure, a preclinical animal model of MDD, has been shown to attenuate serotonin responses in the CA1 region (Karten et al., 1999). Consequently, long-term overexposure to stress and cortisol, known occurrences in depression (O'Keane et al., 2012), may result in CA1 changes and limbic serotonergic system disturbance.

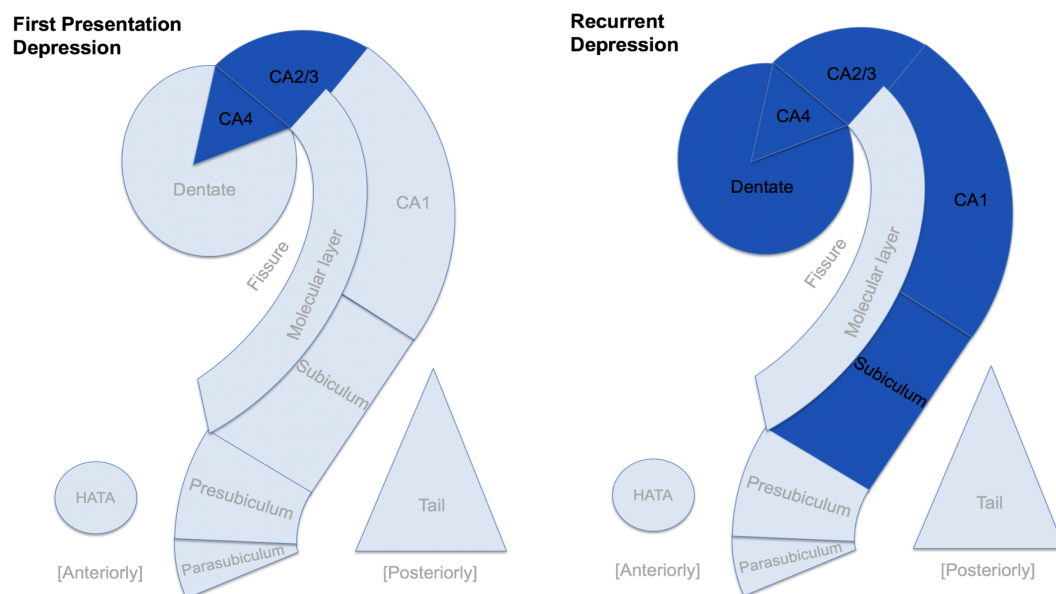


Figure 3.5 First presentation and recurrent depression substructures in MDD. Schematic cross-sections of left hippocampus showing substructures reduced in first presentation depression and also in recurrent depression. Note the greater number of substructures involved in the recurrent depression, suggesting a possible extension of disease process in depression. CA, cornu ammonis; HATA, hippocampal amygdalar transition area.

CA2-4 involvement in first presentation and further extension with chronicity

The CA2/3 and CA4 regions individually demonstrated significant volume reduction in first presentation patients (Figure 3.5.). Combining them together to form an exploratory CA2-4 composite measure further augmented the significance of this region (Table 3.6. Figure 3.6.), especially in FPD ($p=0.0013$). This suggests a potential locus across these two substructures for initial hippocampal pathology in MDD. CA1 also displayed a distinct trend in this direction ($p=0.021$) but did not survive strict FDR correction. These results imply **that the classic CA hippocampal subfields (and CA2-4 in particular) may be impacted early in the disease process**, as is also suggested by the restrictive HP total hippocampal volume measure. Conversely, the left dentate gyrus, subiculum and tail showed volume reductions only in the recurrent group. This suggests that these substructures may be recruited later on as depression progresses from first presentation into chronicity (Figure 3.5). This, importantly, implies that MDD displays the characteristics of a disease process, and hints at how early intervention may help prevent further extension of pathology throughout the hippocampus.

Differences between FPD and RD groups in this thesis are broadly consistent with the premise that the chronicity of depression has an inverse relationship with hippocampal volumes (McKinnon et al., 2009, Sheline et al., 1999, Cobb et al., 2013, Gerritsen et al., 2011).

3.4.5. HYPOTHESIS 5: EXPLORATORY COMPOSITES CAN PROVIDE FURTHER INFORMATION ABOUT MDD THAN JUST THE INDIVIDUAL COMPUTER SUBSTRUCTURES

Additional exploratory composite measures were used to further investigate the volumes of anatomical sub-regions within the hippocampal complex (Table 3.6., Table 3.7. and Figure 3.6.). The CA4 lies within the hilum of the dentate and can be considered part of the dentate (Amaral, 1978). Combining the dentate with CA4 yielded significant volume reduction ($p=0.0004$) in MDD on the left. This finding was

maintained across both first presentation and recurrent groups. Using this composite measure yielded a statistically significant volume reduction on the right side in depression ($p=0.03$). Conversely, a measure removing CA₄ from the other CA regions (i.e. a genuine cornu ammonis only region; CA only with no dentate components, see Chapter 1.3.1 and Chapter 1.3.2 for further explanation) was calculated by adding the volumes of CA₁₋₃. This composite when analysed produced even more significant differences between depressed and controls ($p=0.0003$) on the left, with reductions being found across both depression groups. Finally, by adding dentate and all CA areas together to form a combined dentate/CA measure, the most significant volume reduction overall in depression ($p=0.0001$) was seen, with the differences driven by both first presentation and recurrent groups.

The exceptional significance of the volume changes found in these exploratory composites and also in the restrictive hippocampus proper (CA₁₋₄ regions) reinforces the importance of the left CA and dentate regions as key substructures in depression. Together these regions correspond to the path of the classic trisynaptic circuit, the fundamental hippocampal computational unit (Andersen, 1975, Stepan et al., 2015). This circuit has important roles in the memory functions (Rolls, 2016) of pattern separation and completion as well as incorporating the hippocampal locus of adult neurogenesis (Schoenfeld et al., 2017), processes that are postulated to be disrupted in MDD. It is also a region involved both in cortisol and serotonin responses, key disturbances of both also being hypothesised in the pathogenesis of depression (Frodl et al., 2014, O'Keane et al., 2012, Joels et al., 2004, Delgado, 2000).

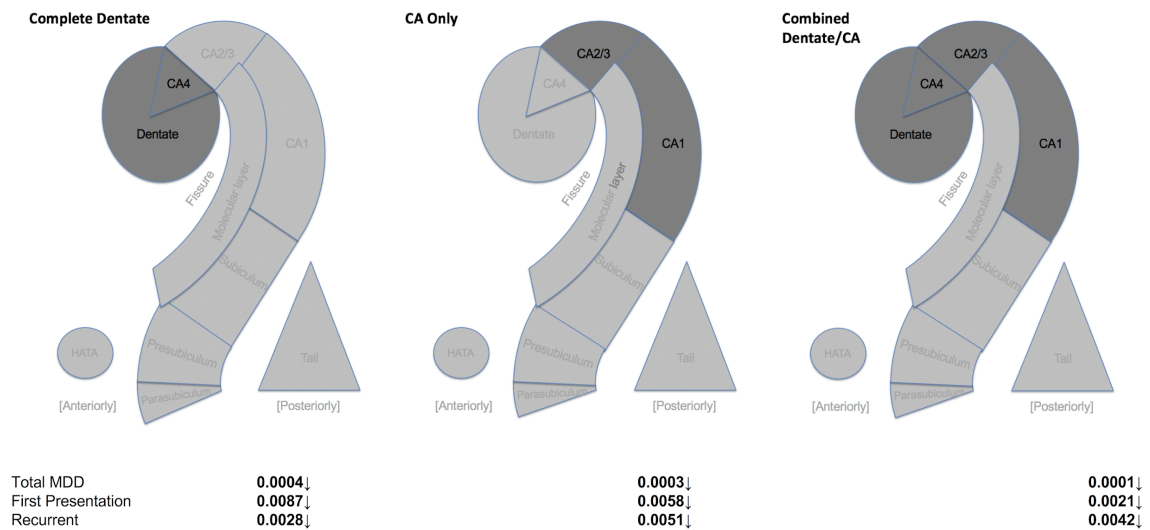


Figure 3.6. Exploratory hippocampal composite measures. Schematic cross-sections of left hippocampal anatomical composite measures. See Table 3.2. for further details on composite assembly. Note that combining all CA and dentate regions into one composite generates a measure with the most significance overall in depression ($p=0.0001$). This highlights the particular importance of the CA and dentate region in depression, regions corresponding to the trisynaptic circuit. CA, cornu ammonis; HATA, hippocampal amygdalar transition area; MDD, major depressive disorder.

This supports the hypothesis that the principal hippocampal pathophysiology in depression is confined within classic principal hippocampal loci only, confirming extensive preclinical research implicating the CA and dentate regions in stress and emotional regulation (Samuels et al., 2015, Samuels and Hen, 2011, Kim et al., 2007, Heine et al., 2004, Rolls, 2016). Exploratory composites may aid further analysis of *FreeSurfer* generated hippocampal substructures.

3.4.6. STRENGTHS AND LIMITATIONS

There are recent moves towards developing a harmonized whole hippocampus segmentation protocol (Bocchetta et al., 2015, Boccardi et al., 2015). The findings described in this chapter suggest a novel method of assembling substructures according to anatomical sub-region definitions and that having such distinct hippocampal definitions is necessary for the rational interpretation of neuroimaging findings in MDD. Other groups using *FreeSurfer* 6.0 have used the automatically generated *FreeSurfer* total hippocampal volume (equivalent to my HE measure) only. As can be seen here, additional valuable information on hippocampal volumes can be harvested by using more distinct hippocampal volumes. The combination of two MRI modalities (T1 and T2-FLAIR) allowed increased accuracy and resolution of the automated technique by providing an additional contrast. This allows superior substructure demarcation compared to T1 alone. This superior discrimination of substructures has previously been shown in an Alzheimer's disease cohort (Iglesias et al., 2015). At the time of writing this thesis, this study is the first to demonstrate substructure differentiation using combined T1 and T2 imaging in any other condition apart from Alzheimer's disease. T1-only differences were also calculated but not considered in this chapter. Between group T1-only depressed and controls can be found in Appendix 4.

Some limitations need to be considered. The average duration of depression in the FPD group was 10 months, with 73% being treated with antidepressants at the time of scanning. 87% of the total depressed cohort were taking some antidepressant

medication at the time of scanning. Ideally, a FPD cohort would consist of treatment-naive patients only, but most of the patients had treatment from their family doctor prior to referral. Thus, I was thus unable to specifically assess the effects of medication on hippocampal substructural volumes due to power constraints. Sex-specific changes have been found in previous hippocampal research (Videbech and Ravnkilde, 2004, Frodl et al., 2002, Kronmüller et al., 2009, Yang et al., 2017). In this study, sex and age were covariates initially and the direct assessment of the effect of sex and age were neutralised as possible confounders (along with eTIV) going forward. Therefore it was impossible to investigate age and sex-specific differences in this study. Automated segmentation has been argued to have reduced interpretation and validity precision compared to expert manual segmentation (Dill et al., 2015). Current automated protocols can differ considerably from each other in terms of image processing, substructure terminology, regional boundaries and segmentation protocols (Yushkevich et al., 2015). Such discrepancies are particularly evident for smaller substructures, and this may be a limitation as *FreeSurfer* 6.0 creates a number of smaller divisions such as the parasubiculum and HATA. The previous iteration of *FreeSurfer* (5.3) has been widely criticised because the computed substructures were not always in line with volumes from anatomical and histological studies (Wisse et al., 2014). In particular, it has been suggested that in the previous version, portions of the large CA1 region are redistributed to the neighbouring CA2/3 resulting in the CA1 region rendered smaller than in anatomical studies (Schoene-Bake et al., 2014). This situation seems to have been addressed in *FreeSurfer* 6.0 (Iglesias et al., 2015) with CA1 volumes now more in line with histological studies for the CA1 and other regions (Walker et al., 2002). Considering the data generated in neuroimaging studies - in this chapter 163 subjects yielded over 3500 individual substructures - automated segmentation is a far more reliable and a much more viable alternative than time and personnel consuming manual segmentation. Finally, even though this study had FPD and RD groups, disease progression cannot be definitively inferred based on such a cross-sectional case control sample. Correlation does not equal causation in this case. The patients and controls in this study are being followed up and will be re-scanned as part of a longitudinal study in the future.

3.5. CONCLUSION

In conclusion, using advanced high quality automated hippocampal segmentation I found patterns of differences across principal hippocampal substructures in a group of patients with MDD, compared to a healthy control group. Consistent with the literature, the left hippocampus was found to be smaller in MDD. This volume difference was found irrespective of hippocampal definition and driven by chronicity. The more restricted the hippocampal definition, the greater the change in depression. No changes were found among the more peripheral hippocampal substructures. This confirms preclinical research identifying the importance of core hippocampal substructures in the pathophysiology of depression and stress. In particular, CA1 volume findings were compelling because of lateralization of volume differences to the left only and because the CA1 volume measure was predictive of MDD duration. The uniqueness of this finding suggests that left CA1 volumes may represent a marker of depressive illness. The initial first presentation involvement of CA2/3/4 regions and additional extension of pathology into adjacent substructures with chronicity suggest a potential MDD disease process. The findings have important clinical implications and reinforce the need for effective early therapeutic intervention in MDD to try and halt possible progressive hippocampal damage.

CHAPTER 4

THE AMYGDALA:

A CENTRAL (AND POSSIBLY MEDIAL) ROLE IN DEPRESSION?

"The workings of the amygdala and its interplay with the neocortex are at the heart of emotional intelligence."

Dr Daniel Goleman.

Emotional Intelligence, 1996

4.1. INTRODUCTION

The defining experience in Major Depressive Disorder (MDD) is of an emotional change where the affected person becomes persistently sad and/or unable to experience pleasure. The other changes in MDD can be grouped into cognitive difficulties and somatic state changes, such as anxiety and circadian rhythm disturbances. The experiential aspects of MDD theoretically map onto brain limbic circuitry, in particular the hub limbic structures of the hippocampus and the amygdala (Kaiser et al., 2015, Pruessner et al., 2010) (See Chapter 1.2.).

Most of the neuroimaging literature in MDD has examined the hippocampus and a consensus view has emerged that there is a reduction in overall hippocampal volume in state MDD (Kempton et al., 2011, Cole et al., 2011). Hippocampal pathology is consistent with the key cognitive difficulties experienced in MDD: impairments in short term memory, in autobiographical memory formation, and impaired attention (Kohler et al., 2015). In the previous chapter (chapter 3), using detailed and automated assessment of hippocampal substructures, I found that the core hippocampal neuronal layers involved in coding memories - the cornu ammonis (CA) - are specifically impacted in MDD, with the left CA1 in particular reducing with increasing chronicity of the disorder. This supports evidence from the psychology literature that autobiographical memory impairments characterise state MDD and predict recurrence (Solomon et al., 2000, Sumner et al., 2010).

The central experience in depression, i.e. that of an unremitting negative mood, must involve brain emotional circuitry. The amygdala - densely interconnected to the hippocampus - is the emotional hub of the limbic network and confers emotional tone and emotional responses to sensory inputs and to memory (Phelps and LeDoux, 2005) (see Chapter 1.2.). There is a large literature dating from the seminal work in the 1930s by Klüver and Bucy demonstrating that amygdala activation is associated with the experience of negative emotional states (Klüver and Bucy, 1939, Gallagher and Chiba, 1996). Possibly the most convincing finding from human studies is that

direct stimulation of the amygdala, and in particular the right amygdala, with depth EEG electrodes during the pre-operative work-up for epilepsy surgery induces negative emotional feeling states (Lanteaume et al., 2007). Amygdala pathology in MDD fits with the emerging evidence that amygdala anatomy may be altered by stress experienced during childhood development and that this may be a harbinger of adult mood disorders (Tottenham et al., 2010, Connolly et al., 2017, Wen et al., 2017).

Most of the human evidence for amygdala involvement in MDD comes from neuroimaging studies that examine emotional limbic circuitry. The amygdala is connected to higher emotional regulatory networks such as the medial prefrontal cortex that are largely inhibitory in nature (Chapter 1.2). Neuroimaging studies have shown that the normal “brake” on amygdala firing from prefrontal areas is disrupted in MDD (Carballedo et al., 2011). Fewer studies have examined amygdala volume in MDD and overall there is a disappointing inconsistency in the findings, with some showing a reduced (van Elst et al., 2000), some an increased (Saleh et al., 2012), but most an unchanged, (Zavorotnyy et al., 2018, Sandu et al., 2017, Arnone et al., 2012, Hamilton et al., 2008, Keller et al., 2008) amygdala volume. One group have reported that heightened negative affect is associated with increased amygdala and reduced prefrontal volumes in healthy adults (Holmes et al., 2012). It is likely that inconsistent findings on amygdala volume reflect the inconsistent, and technically difficult, methods for measuring this complex structure. Rather than being a unitary structure, the amygdala is formed from a collection of interconnected substructures (nuclei) that relay information from multiple brain areas, primarily cortical and sensory, and connect to, among others, emotion-making systems in the body. The nuclei can be clustered into three broad groups; laterobasal, medial and superficial (Heimer et al., 1999) (see Chapter 1.4.1). Broadly speaking, the main cortical inputs go to the lateral nuclei, while outputs from the amygdala emerge primarily from more medial structures, i.e. the medial and central nuclei (Heimer et al., 1999).

A substantial efferent pathway from the centromedial amygdala relays to the hypothalamus: a final common brain exit point from which visceral function is controlled through the autonomic nervous system (ANS), and homeostatic equilibrium is controlled through the hypothalamic-pituitary-adrenal (HPA) axis (Barbier et al., 2018, Van de Kar and Blair, 1999). The amygdala-hypothalamus connection is central to the experience of emotions because the hypothalamus regulates ANS function, and hence body feeling states (Beissner et al., 2013, Giardino et al., 2018). It is likely that the balance of the sympathetic-parasympathetic systems of the ANS determines subjective visceral emotional states in humans, with sympathetic drive leading to states of arousal and fear, and parasympathetic drive mediating “rest and digest” feeling states (Beissner et al., 2013, Gallagher and Chiba, 1996). Findings from a recently published study in mice led the authors to propose a model of fine-tuned amygdala control of lateral hypothalamic nuclei that could result in opposing emotional states (Giardino et al., 2018).

Efferent pathways from the amygdala also control the body's homeostatic system, the HPA axis (Kirouac, 2015). The paraventricular and the supraoptic nuclei in the hypothalamus that produce and secrete corticotrophic releasing hormone (CRH) both receive stimulatory inputs from the medial amygdala (Herman et al., 2005). The central nucleus also modulates the HPA-stress axis, but more so in response to physical stressors than psychological stressors (Dayas et al., 1999). See Figure 4.2. for a schematic representation. Similar to the hippocampus, the amygdala has glucocorticoid receptors that mediate feedback inhibition of hypothalamic CRH secretion. Overdrive in the HPA axis has been established in MDD (O'Keane et al., 2012), and is generally accepted now as resulting from a reduced hippocampal brake on HPA axis drive (Frodl and O'Keane, 2013). Impaired hippocampal inhibition of the HPA axis, leading to the over arousal and circadian rhythm disturbances experienced in depression, has been attributed to hippocampal atrophy (Sapolsky et al., 1991), and in more recent years to poor glucocorticoid receptor function in the hippocampus (O'Keane et al., 2012). The hippocampal-HPA hypothesis has only been weakly supported by human research findings. Specifically, there have been no consistent

associations found between overdrive in the HPA axis and hippocampal volume measures (Frodl and O'Keane, 2013).

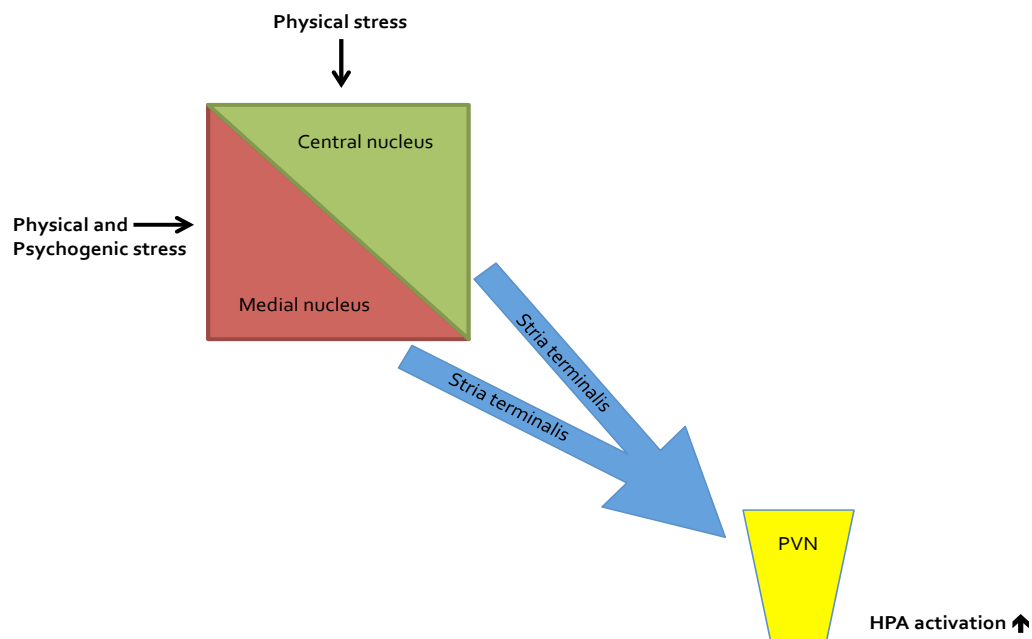


Figure 4.1. Centromedial activation of the HPA axis. Physically stressful inputs are processed through the central and medial nuclei, whereas as psychogenic inputs are processed through the medial nucleus. Both increase cortisol secretion through HPA activation. HPA, hypothalamic-pituitary-adrenal axis; PVN, paraventricular nucleus of the hypothalamus.

Amygdala and nuclear boundaries can be difficult to define and until recently consistent estimation of nuclei size was not possible. In this chapter amygdala measures are examined at a substructure (nuclear) level, as the hub of emotional and body arousal systems. In this study I used advanced software technology, *FreeSurfer* 6.0, which allows automated evaluation of the detailed nuclei to examine, for the first time, amygdala sub-structure volumes in a group of patients with MDD and healthy controls. Using salivary cortisol samples collected from a subset of patients and analysed by two other PhD students from the REDEEM group (Dr Kelly Doolin and Dr Chloe Farrell) and at Manchester University, I examined the relationships between amygdala measures and the stress response using the cortisol awakening response (CAR) as a measure of HPA activity. These CAR measures were also compared to whole hippocampal volumes and substructures.

Amygdala and nuclear boundaries can be difficult to define and until recently consistent estimation of nuclei size was not possible. In this chapter amygdala measures were examined at a substructure (nuclear) level, as the hub of emotional and body arousal systems. In this study, similar to the previous chapter, I used advanced software technology, *FreeSurfer 6.0*, which permits automated evaluation of the detailed amygdala nuclei. To my knowledge this is the first time that amygdala substructure volumes have been examined in a group of patients with MDD and healthy controls. Similar to the hippocampal study, I used novel composite measures to aid my analysis. Using salivary cortisol samples collected from a subset of patients and analysed by two other previous PhD students from the REDEEM group (Dr Kelly Doolin and Dr Chloe Farrell) and also by Professor Brian Keevil at Manchester University, I examined the relationships between amygdala measures and the stress response using the cortisol awakening response (CAR) as a measure of HPA activity. These CAR measures were also compared to whole amygdala volumes and substructures to evaluate the relationship between HPA activity, amygdala volumes and depression.

4.1.1. HYPOTHESES

My **hypotheses** were that:

1. amygdala volume differences exist in MDD at a nuclear level but not at a whole amygdala level,
2. amygdala volume differences in MDD are predominately located in the centromedial nuclei,
3. amygdala asymmetry occurs in MDD, reflecting the difference in emotional response (right – negative affect, left – positive affect), and
4. the CAR, as a measure of chronic HPA axis overactivity, is negatively correlated with whole and nuclear amygdalar volumes in MDD.

4.2. METHODS

The methods used for this study are described in detail in Chapter 2.2., Chapter 2.3., Chapter 2.4. and Chapter 2.5., Chapter 2.8. and Chapter 2.9. A brief summary description is given below.

Eighty people with Major Depressive Disorder (MDD) were compared to eighty-three healthy controls without MDD. All participants completed the MINI (Mini International Neuropsychiatric Interview for DSM IV (American Psychiatric Association, 1994) and Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960). All patients required a current MINI diagnosis of MDD with a HAM-D greater than 20. Controls were required to have no active or previous MINI diagnosis and a HAM-D less than 8. A subsample of participants were also investigated for the CAR.

4.2.1. MRI ACQUISITION

All data was acquired on a Philips (Best, Netherlands) Intera Achieva 3.0 Tesla MR system (32-channel head coil) at Trinity College Institute of Neuroscience, Dublin.

T1. 180 axial high-resolution T1-weighted anatomical images were acquired (T1W-IR1150 sequence, TE = 3.8 ms, TR = 8.4 ms, FOV 230 mm, $0.898 \times 0.898 \text{ mm}^2$, in-plane resolution, slice thickness 0.9 mm, flip angle $\alpha = 8^\circ$).

Unlike the hippocampus, T2 images are not required for *FreeSurfer* 6.0 automated segmentation of the amygdala. This feature now available in the developmental version of *FreeSurfer* 6.0, which in addition to the aforementioned hippocampal subfield module featured in the stable release, the amygdala analysis now offers similar functionality and divides the structure in nine sub nuclei.

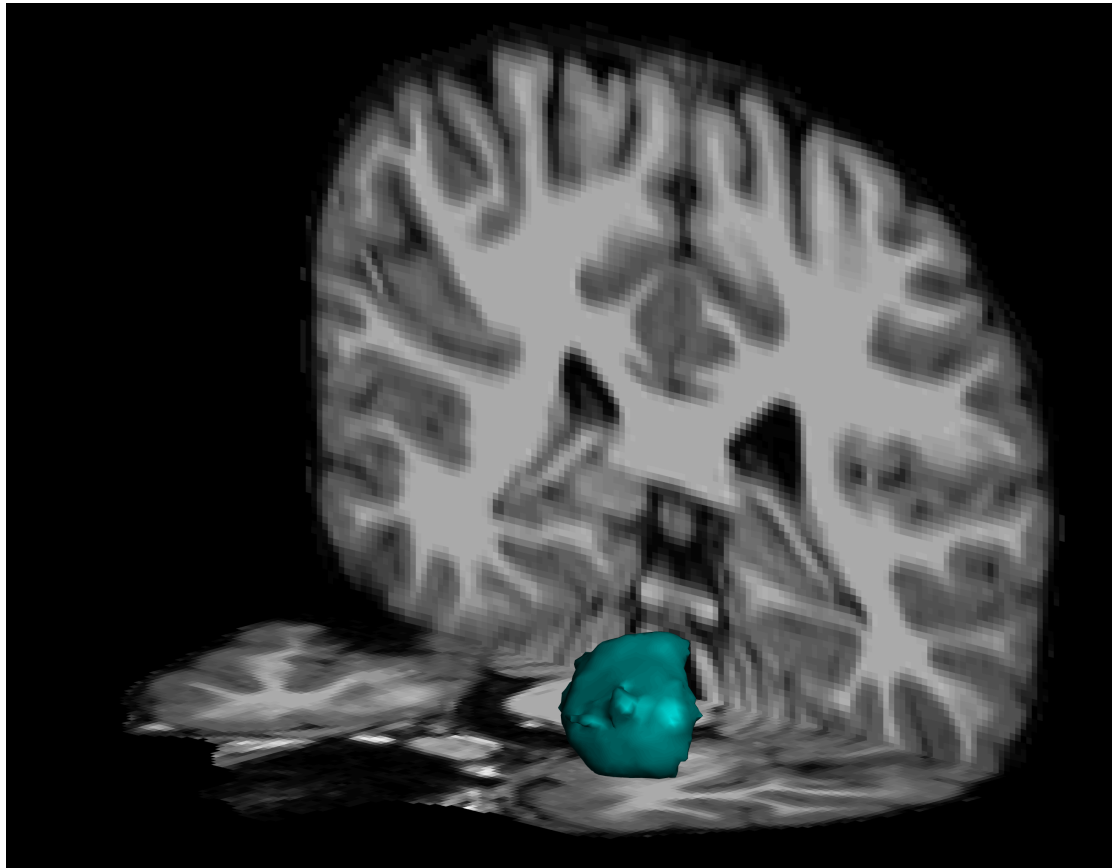


Figure 4.2. *FreeSurfer* generated whole amygdala. *FreeSurfer* generated left whole amygdala sitting in combined axial and coronal T1 image. The left amygdala is seen in petrol-blue colour.

4.2.2. CORTISOL MEASURES

Salivary cortisol was measured in a subsample of participants. Saliva samples were collected in Salivette® tubes (Sarstedt) by the study participants at three time points after wakening (0, 30, and 60 minutes). Centrifuging the Salivettes extracted the saliva and this saliva was aliquoted into microtubes and subsequently frozen at 80 degrees.

Liquid Chromatography – Mass Spectrometry (LC-MS) was performed at the University of Manchester in the Department of Clinical Biochemistry by members of Professor Brian Keevil's laboratory at the University Hospital of South Manchester. This consisted of 3 phases (Owen et al., 2010); sample preparation, chromatography using a Shimadzu Prominence LC system (Shimadzu, Milton Keynes, UK), and mass

spectrometry using a Quattro MicroTM tandem mass spectrometer (Waters, Manchester, UK). See Chapter 2.8 for more detail.

Data cleaning was carried out by eliminating values that were above or below two standard deviations from the mean; however there were no outliers found in the morning time point LCMS data. Cortisol awakening response (CAR) was calculated using the Area Under the Curve (AUC_i) with respect to increase from baseline (Fekedulegn et al., 2007). The equation is as follows:

$$[(T_0 \text{ cortisol} + T_{30} \text{ cortisol})/2 \times \text{time difference} + (T_{30} \text{ cortisol} + T_{60} \text{ cortisol})/2 \times \text{time difference}] - [T_0 \text{ cortisol} \times \text{total time difference}].$$

4.2.3. IMAGE ANALYSES

Cortical reconstruction and segmentation was performed using the developmental version (released August 2018) of *FreeSurfer* 6.0 image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>) (Fischl, 2012a, Fischl and Dale, 2000). The technical details of these procedures are described elsewhere (Fischl et al., 2002b, Desikan et al., 2006, Fischl et al., 1999, Fischl et al., 2004, Reuter et al., 2012, Dale et al., 1999). *FreeSurfer* interrogates T1 contrast differences between substructures using previously defined in-vivo and ex-vivo amygdala atlases to determine substructure characteristics. By selecting the 3T MRI flag in *FreeSurfer* the procedure was optimized. 9 substructures were computed (Table 4.1). See also Chapter 2, Table 2.4. for details of anatomical landmarks used for automated segmentation.

COMPOSITE MEASURES

Computed substructure volumes were summed together to generate composite measures. Three amygdala composites were generated to correspond with the three anatomic-functional groups discussed in Chapter 1; superficial, laterobasal and centromedial (Johnston, 1923, Heimer et al., 1999). A further whole amygdala

volume was generated by summing all computed outputs. See Table 4.1 and Chapter 2, Table 2.6 for details of summed components.

4.2.4 STATISTICAL ANALYSES

All extracted subfield volumes were systematically inspected visually, and measures exported to SPSS24 (<https://www.ibm.com/analytics/us/en/technology/spss/>). An initial global model was applied using a mixed-method-repeated measured Analyses of Variance (ANOVA) with hemisphere (left and right) and nuclei (nine subdivisions) as within-subject factors and group (Depressed and Controls) as the between-subject factor. All interaction effects were also carefully examined. This initial design was used to investigate group-wise differences in substructure/composite volumes and across all substructures/composites and hemisphere (left/right) of the amygdalae. To clarify any main driving effects identified and complex interaction effects, additional repeated measures ANOVA's were computed for each nucleus independently. Finally, post-hoc Analyses of Covariance (ANCOVA) were used to compare between-group differences (controls v depressed only) for each substructure/composite and hemisphere independently. Age, sex and eTIV were entered as covariates throughout. Multiple Comparison Correction (MCC) was performed using False Discovery Rate (FDR) correction (Benjamini, 2010) . Finally, linear regression was employed to examine two additional components, namely;

1. Group classification (Controls or Depressed): A two-step binary logistic regression model was used to test the ability of the model to identify group classification based on bilateral volumetric quantifications of the central and medial nuclei while controlling for the effects of age, sex and eTIV. The first step of the model included age, sex and eTIV as covariates while the second step of the model included bilateral central and medial nuclei volumes as predictors.
2. Chronicity of Depression: A two step hierarchical linear regression model was used to investigate the ability of the model to significantly predict disease

chronicity based on duration of illness. The difference between left and right central and medial nuclei volumes (i.e. the delta central nucleus and medial nucleus volumes) were calculated and log transformed to yield a standardized measure. A two-step hierarchical linear regression model was used with disease duration (transformed) entered as the dependent variable. The first step of the model included age, sex and eTIV as covariates. Right-left differences in the central and medial nuclei were entered in the second block of the model and the predictive ability of substructures on disease duration was thus examined (see Chapter 2).

AMYGDALA SYMMETRY

The symmetry of all nuclei volumes were examined to assess potential group differences in each nucleus. Right-left symmetry was assessed using transformed delta volume differences (i.e. the difference between right and left volumes) via a series of nucleus independent ANCOVA's correcting for age, sex and eTIV.

CORTISOL

The CAR was measured using two calculations; the area under the curve with respect to increase from baseline (AUC_i) and the area under the curve with respect to ground (AUC_g) (Doolin et al., 2017, Fekedulegn et al., 2007) (see Figure 4.5. for example and Chapter 2 for further details). Split-group partial correlations were performed to examine the relationship between nuclei volume measures and cortisol awakening response measures, AUC_i and AUC_g for each hemisphere.

Age, sex and eTIV (total intracranial volume) were treated as covariates throughout all analyses. Multiple Comparison Correction (MCC) was performed using False Discovery Rate (FDR) correction (Benjamini, 2010)

Substructure
Lateral
Basal
Accessory Basal
Paralaminar nuclei
Central
Medial
Anterior Amygdala Area
Corticoamygdaloid Transition Area
Cortical

Table 4.1. Computed amygdala substructures

Composite
Whole amygdala
Laterobasal
Centromedial
Superficial

Table 4.2 Amygdala composites

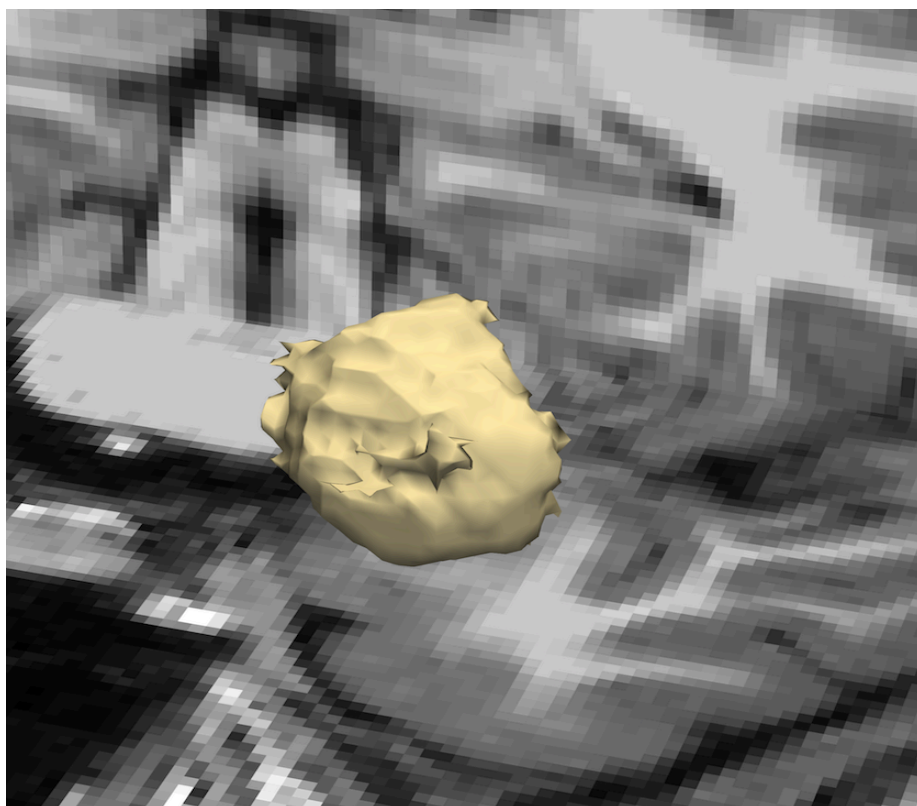


Figure 4.3. *FreeSurfer 6.0* whole amygdala image. Close up of whole left amygdala in a T1 axial and coronal image.

4.3. RESULTS

No significant between-group differences between controls and total depressed or between controls and FPD were found for age, sex or handedness. However, the RD group was different from both the controls and FPD group, with the RD having a higher proportion of females as well as being significantly older (10-12 years older). While HAM-D was higher in patients compared to controls, no difference was found between FPD and RD groups. The average duration of depression in FPD and RD was 10 months and 60 months respectively. All RD patients and 73% of FPD patients (27/37) were taking antidepressants at the time of scanning. See Table 4.3a. A subset of forty-two patients and twenty-five controls had sufficient salivary cortisol samples (three timepoints @ 0, 30, 60 mins post waking) to estimate the cortisol awakening response. See Table 4.3b.

	Group		Con v Dep
	Con N=83	Dep N=80	<i>p</i> -value
Age: Mean(SEM)	31.5(1.4)	34.5(1.4)	0.13
Range (years)	16-64	17-64	-----
Male/Female (%M)	34/49, 41%	23/57, 29%	X ² : 0.14
Handedness (R/L)	75/8	73/7	NS
HAMD* (SEM)	1.3(0.4)	22.2(0.4)	1.10E-75
MDD months**	-----	29(4)	-----

Table 4.3a. Demographic data for amygdala volumetrics. Con, controls; Dep, depressed; HAM-D, Hamilton Depression scale; M, male; MDD, major depressive disorder; R/L, right or left handed; SEM, standard error of the mean.

	Group		Con v Dep
	Con N=25	Dep N=42	<i>p</i> -value
Age: Mean(SEM)	31.26(2.1)	35.6(1.9)	0.1
Range (years)	18-60	17-62	-----
Male/Female (%M)	7/18, 37.3%	13/29, 43.3%	X ² : 0.065
Handedness (R/L)	23-Fe	38/4	NS
HAMD* (SEM)	2.3(0.7)	23.2(0.6)	1.60E-75
MDD months**	-----	30(7)	-----

Table 4.3b. Demographic data for CAR. Con, controls; Dep, depressed; HAM-D, Hamilton Depression scale; M, male; MDD, major depressive disorder; R/L, right or left handed; SEM, standard error of the mean.

4.3.1. AMYGDALA VOLUMETRICS

See Table 4.4. The initial global mixed-model repeated measures ANOVA with age, sex and eTIV as covariates generated no significant main effect for group. There was no main effect for hemisphere, but a significant group x hemisphere interaction (Greenhouse-Geisser, $F(7.11, 1\text{dof})$, $p=0.009$, partial $\eta^2=0.05$, power=0.75) was found. A main effect for sub-nucleus (Greenhouse-Geisser $F(14.42, 8\text{dof})$, $p=0.00001$, partial $\eta^2=0.05$, power=0.75) and group x hemisphere x substructure interaction (Greenhouse-Geisser $F(4.37, 2.318\text{dof})$, $p=0.01$, partial $\eta^2=0.03$, power=0.75) was also identified.

Amygdala Structure / Composite	Within Subject	Between Group Effect	Interaction (Group x Nucleus)
	F stat (dof), p-value		
Lateral_nucleus	0.01(1), 0.91	1.26(1), 0.26	7.4(1), 0.007
Basal nucleus	0.97(1), 0.33	0.02(1), 0.88	6.1(1), 0.015
Accessory basal nucleus	0.20(1), 0.66	0.001(1), 0.99	1.88(1), 0.17
Anterior amygdaloid area	0.37(1), 0.55	0.14(1), 0.71	3.02(1), 0.08
Central_nucleus	0.01(1), 0.91	0.01(1), 0.94	9.93(1), 0.002
Medial nucleus	2.12(1), 0.15	5.70(1), 0.02	4.58(1), 0.034
Cortical nucleus	0.27(1), 0.60	0.01(1), 0.92	0.24(1), 0.62
Corticoamygdaloid transition area	0.12(1), 0.73	0.05(1), 0.83	0.01(1), 0.95
Paralaminar nucleus	5.26(1), 0.02	2.30(1), 0.13	7.60(1), 0.007
Whole amygdala	0.13(1), 0.72	0.33(1), 0.60	6.62(1), 0.011
FDR Threshold for MCC across all nine Nuclei = 0.015			
FDR Threshold for MCC across four composites = 0.011			

Figure 4.4. Repeated measure ANOVAs correcting for age, sex and eTIV. Dof, degrees of freedom; FDR, false discovery rate; MCC, multiple comparison correction.

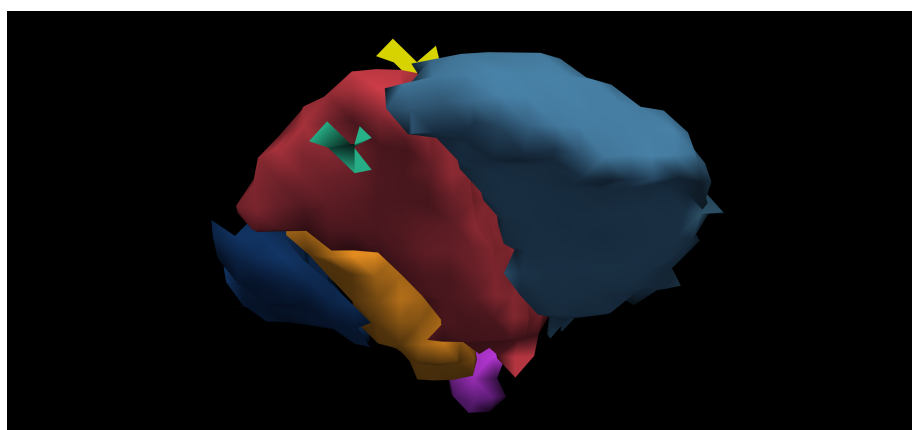


Figure 4.4. FreeSurfer 6.0 amygdala substructures representative image. Different colours represent specific nuclei; green, medial; dark blue, corticoamygdala transitional area; orange, accessory basal; red, basal; purple, central; yellow, anterior amygdaloid area; light blue, lateral

SECONDARY REPEATED MEASURES ANCOVA'S

See Table 4.5., Table 4.6. and Table 4.7. The secondary series of repeated measure ANCOVA's for each independent nucleus identified a consistent pattern of change across four of the nine nuclei ($p < 0.05$). Post FDR correction for multiple comparisons identified the most significant difference in the right central nucleus ($p = 0.002$) (refer to Table 4.6.), with the right medial nucleus difference also just above the FDR threshold (Table 4.6.). The amygdala as a whole also showed a significant group x nucleus interaction effect ($p = 0.011$) and there were also focused centromedial and laterobasal composite changes identified (Table 4.7.). Further hemisphere-independent post hoc analyses identified trend level between-group differences in the left central nucleus ($p = 0.059$), uncorrected paralaminar nucleus (0.018), and right lateral nucleus ($p = 0.033$), with significant right medial nucleus ($p = 0.002$) increases surviving stringent FDR MCC correction (Table 4.5. and 4.6.). Composite analysis showed a no significant change in whole amygdala volumes. The right centromedial group was increased ($p = 0.01$). See Table 4.6.

	Nucleus	Left		
		Cons v MDD	MDD (mm ³)	Controls (mm ³)
		p-value, partial eta ²	Mean (SEM) 95% CI	Mean (SEM) 95% CI
Nuclei	Lateral nucleus	0.91, 0.0005	643.53 (5.83,632-655)	642.59 (5.79,631-654)
	Basal nucleus	0.27, 0.008	417.06 (3.38,410-424)	422.39 (3.40,416-429)
	Accessory basal nucleus	0.57, 0.002	252.31 (2.21,248-257)	254.11 (2.12,250-258)
	Anterior amygdaloid area	0.25, 0.009	52.70 (0.68,51-54)	53.81 (0.67,53-55)
	Central nucleus	0.059, 0.024	43.27 (0.79, 41.70-44.83)	45.40 (0.78, 43.86-46.93)
	Medial nucleus	0.28, 0.008	20.68 (0.64, 19.41-21.96)	19.70 (0.64, 18.44-20.95)
	Cortical nucleus	0.75, 0.001	24.97 (0.44,24.10-25.84)	24.77 (0.44,23.91-25.64)
	Corticoamygdaloid transition	0.82, 0.001	192.07 (1.80,189-196)	192 (1.8,188-195)
	Paralaminar nucleus	0.018, 0.037	48.77 (0.52,47.74-49.79)	50.52 (0.51,49.51-51.52)

Table 4.5. Left amygdala between group differences for individual substructures following ANCOVA correcting for age, sex and eTIV and also after correcting for multiple comparisons using FDR. Representative volumes are shown in mm³ with standard deviations. Numbers in parenthesis show actual numbers following removal of outliers ensuring all data fell within the 95% confidence interval. Bold text indicates p-values that survived FDR correction. ANCOVA, analysis of covariance; CI, confidence interval; Con, controls; eTIV, estimated total intracranial volume; FDR, false discovery rate; MDD; major depressive disorder; SEM, standard error of the mean.

	Nucleus	Right		
		Cons v MDD	MDD (mm ³)	Controls (mm ³)
		p-value, partial eta ²	Mean (SEM) 95% CI	Mean (SEM) 95% CI
Nuclei	Lateral nucleus	0.033, 0.03	668.18 (5.05,658-678)	652.81(5.02,643-663)
	Basal nucleus	0.37, 0.005	434.95 (3.18,429-441)	430.91(3.20,425-437)
	Accessory basal nucleus	0.57, 0.002	261.46 (2.20,257-266)	259.69 (2.12,255-264)
	Anterior amygdaloid area	0.69, 0.001	56.73 (0.75,55-58)	56.30 (0.74,55-58)
	Central nucleus	0.095, 0.019	48.86 (0.96, 46-96-50.76)	46.58 (0.94,44.71-48.44)
	Medial nucleus	0.002, 0.064	23.98 (0.61, 22.19-24.60)	20.64 (0.60, 19.45-21.82)
	Cortical nucleus	0.87, 0.0005	26.87 (0.41,26.05-27.68)	26.96 (0.4126.15-27.76)
	Corticoamygdaloid transition	0.87, 0.0005	193.30 (1.74,190-197)	193.00 (1.72,190-196)
	Paralaminar nucleus	0.88, 0.0005	50.84 (0.44, 49.98-51.70)	50.93 (0.43, 50.08-51.77)

Table 4.6. Right amygdala between group differences for individual substructures following ANCOVA correcting for age, sex and eTIV and also after correcting for multiple comparisons using FDR. Representative volumes are shown in mm³ with standard deviations. Numbers in parenthesis show actual numbers following removal of outliers ensuring all data fell within the 95% confidence interval. Bold text indicates p-values that survived FDR correction. ANCOVA, analysis of covariance; CI, confidence interval; Con, controls; eTIV, estimated total intracranial volume; FDR, false discovery rate; MDD; major depressive disorder; SEM, standard error of the mean.

	Composite measure	Cons v MDD	MDD (mm ³)	Controls (mm ³)
		p-value, partial eta ²	Mean (SEM) 95% CI	Mean (SEM) 95% CI
Left	Whole amygdala	0.7, 0.001	1692 (13,1666-1718)	1699 (13,1673-1758)
	Laterobasal	0.57, 0.002	1358 (11,1337-1379)	1367 (11,1346-1388)
	Centromedial	0.65, 0.001	64.25 (1.3,61.67-66.82)	65.10 (1.29,62.57-67.64)
	Superficial	0.97, 0.0005	269.72 (2.4,265-274)	269.86 (2.4,265-275)
Right	Whole amygdala	0.13, 0.015	1696(13,1674-1725)	1734 (12,1710-1758)
	Laterobasal	0.16, 0.014	1412 (10,1393-1431)	1392 (10,1373-1412)
	Centromedial	0.01, 0.043	72.33 (1.42,69.52-75.13)	67.11 (1.40,64.34-69.87)
	Superficial	0.65, 0.001	277.20 (2.4,273-282)	275.64 (2.4,271-280)

Table 4.7. Left and right amygdala between group differences for composites following ANCOVA correcting for age, sex and eTIV and also after correcting for multiple comparisons using FDR. Representative volumes are shown in mm³ with standard deviations. Numbers in parenthesis show actual numbers following removal of outliers ensuring all data fell within the 95% confidence interval. Partial eta² describes effect size (0.01=low, 0.06=moderate, 0.14=large). Bold text indicates p-values that survived FDR correction or moderate to large effect size. FDR for each group is found at the bottom of the table. ANCOVA, analysis of covariance; CI, confidence interval; Con, controls; eTIV, estimated total intracranial volume; FDR, false discovery rate; MDD; major depressive disorder; SEM, standard error of the mean.

AMYGDALA ASYMMETRY

Hemisphere asymmetry ANCOVAs for each nucleus revealed a pattern of change ($p < 0.05$) in three sub-nuclei (basal, central and medial) with the central nucleus ($p = 0.003$, $\eta^2 = 0.07$, observed power 0.86) surviving a reduced FDR threshold of 0.003 for MCC. Whole amygdalae and laterobasal showed similar asymmetry ($p = 0.004$, and $p = 0.009$ corrected) (see Table 4.8.).

		R-L Asymmetry
		(transformed)
	Nucleus/Composite measure	p , (η^2 , power estimate)
Nuclei	Lateral nucleus	0.25 (0.009, .73)
	Basal nucleus	0.047 (0.03, 0.51)
	Accessory basal nucleus	0.076 (0.02, 0.43)
	Anterior amygdaloid area	0.815 (0.001, 0.06)
	Central nucleus	0.003 (0.07, 0.86)
	Medial nucleus	0.014 (0.04, 0.70)
	Cortical nucleus	0.239 (0.009, 0.22)
	Corticoamygdaloid transition	0.855 (0.001, 0.05)
	Paralaminar nucleus	0.287 (0.008, 0.19)
Composite	Whole amygdala	0.004 (0.05, 0.83)
	Laterobasal	0.009 (0.04, 0.75)
	Centromedial	0.107 (0.02, 0.36)
	Superficial	0.164 (0.01, 0.28)

Table 4.8. Right-left asymmetry for individual nuclei and composites following ANCOVA correcting for age, sex and eTIV and also after correcting for multiple comparisons using FDR. Representative volumes are shown in mm³ with standard deviations. Numbers in parenthesis show actual numbers following removal of outliers ensuring all data fell within the 95% confidence interval. Partial η^2 describes effect size (0.01=low, 0.06=moderate, 0.14=large). Bold text indicates p-values that survived FDR correction or moderate to large effect size. ANCOVA, analysis of covariance; eTIV, estimated total intracranial volume; FDR, false discovery rate; R-L, right-left.

4.3.2. HIERARCHICAL REGRESSION

GROUP PREDICTION: HIERARCHICAL BINARY LOGISTIC REGRESSION

As a whole, the prediction model explained between 13.7% (Cox and Snell R^2) and 18.3% (Nagelkerke R^2) of the variance associated with group allocation and correctly identified group membership in 66% of cases compared to the baseline model which explained 50.7% of the total variance. Only two of the independent predictors (left central nucleus and right medial nucleus respectively) made unique statistically significant contributions to the model. The left central nucleus was identified as the strongest predictor with an odds ratio of (Wald 7.32, dof 1, 0.914, $p=0.007$, CI 0.856-0.976) followed by the right medial nucleus with an odds ratio of (Wald 5.49, dof 1, 1.12, $p=0.019$, CI 1.02-1.24) when controlling for age gender and eTIV.

DISEASE DURATION PREDICTION IN MDD: HIERARCHICAL TWO STEP LINEAR REGRESSION

A two-step hierarchical multiple regression model identified the central nucleus delta, or differences between the R and L central nuclei, as a significant predictor variable in MDD explaining an additional 6.4% of the variance in disease duration when age, sex and eTIV were controlled for (R squared change =0.064, F change (2,69) =3.41, $p=0.039$). The final model revealed the central nucleus delta as a statistically significant predictor ($\beta=0.226$, $p=0.029$), and the medial nucleus delta at strong trend level ($\beta=-0.196$, $p=0.059$).

4.3.3. HPA-AXIS AND AMYGDALA VOLUMES

There was a difference between the AUCi in the MDD and controls, with AUCi lower in the MDD group. This was probably due to a higher baseline cortisol in the MDD group, and thus a smaller difference between the baseline and the CAR, indicating relative cortisol hypersecretion in MDD.

Split-group partial correlations revealed a consistent bilateral pattern of negative correlation between sub-nuclei volumes and the CAR AUCi calculation (Table 4.9.). These correlations were more pronounced on the right side and across more nuclear groups. The centromedial volumes were only negatively correlated with the cortisol AUCi on the right side. No correlations were found between the AUCg calculation of the CAR and any amygdala measure.

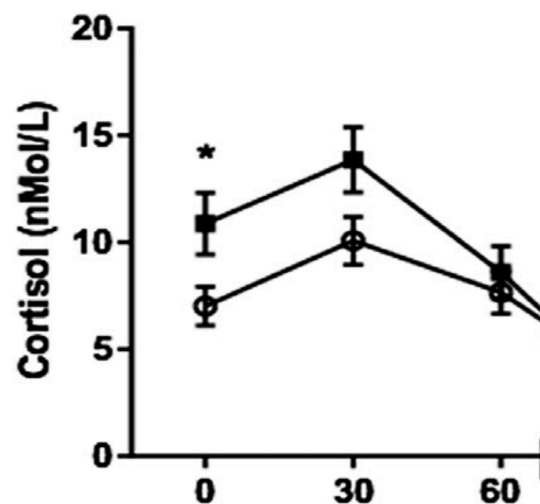


Figure 4.5. Examples of cortisol awakening response curves. X-axis shows time in minutes from wakening, Y-axis shows cortisol concentrations in nMol/L. Note the increase from wakening at 30 mins and return at 60 mins, suggestive of an elastic, reactive CAR. Squares indicate a patient with MDD, circles indicate a control participant. CAR, cortisol awakening response; MDD, major depressive disorder.

Structure	AUCg	AUCi
	p-value, r-stat	
	LEFT	
Lateral	0.176	0.0004, 0.55
Basal	0.824	0.007, 0.43
Accessory basal	0.751	<i>0.024, 0.37</i>
Anterior amygdaloid area	0.084	0.118, 0.26
Central	0.384	0.395, 0.14
Medial	0.36	0.144, 0.25
Cortical	0.509	0.085, 0.28
Cortical amygdalar transition area	0.213	0.121, 0.26
Paralaminar	0.793	0.448
Whole amygdala	0.913	0.001, 0.5
Laterobasal	0.175	0.001, 0.51
Centromedial	0.211	0.197, 0.21
Superficial	0.228	<i>0.046, 0.33</i>
	RIGHT	
Lateral	0.248	0.015, 0.40
Basal	0.412	0.015, 0.39
Accessory basal	0.953	0.005, 0.445
Anterior amygdaloid area	0.755	0.122, 0.26
Central	0.13	0.072, 0.30
Medial	0.057	0.007, 0.43
Cortical	0.293	0.005, 0.45
Cortical amygdalar transition area	0.695	0.008, 0.427
Paralaminar	0.21	0.387, 0.14
Whole amygdala	0.817	0.004, 0.46
Laterobasal	0.987	0.007, 0.43
Centromedial	<i>0.046</i>	0.016, 0.39
Superficial	0.842	0.001, 0.52

Table 4.9. Correlations for CAR measures and left and right amygdala substructures and composites for the MDD group. Only p values and r-stats are shown. FDR was used to correct for multiple comparisons throughout revealing a cutoff for significance of 0.015 across the eighteen nuclei measures and 0.016 across the eight composite measures. Italic text denotes p-values <0.05 but not surviving the FDR threshold. R-stat denotes Pearson's correlation, a measure of effect size: small, 0.10; medium, 0.30; large 0.50. Bold text survives FDR correction. Directionality for all correlations surviving FDR is negative. AUCg; area under the curve with respect to ground; AUCi, area under the curve with respect to increase from baseline; CAR, cortisol awakening response; FDR, false discovery rate; MDD, major depressive disorder.

4.4. DISCUSSION

Whole amygdala volume was not changed in major depressive disorder (MDD). However, throughout the analysis, the central and medial nuclei demonstrated differences in MDD compared to controls. The medial nucleus was enlarged on the right side in MDD. The increase in amygdala asymmetry in depression was noticeably driven by the relative increase in right central and medial nuclei in MDD. Both these nuclei provide the dominant amygdalar output to the autonomic nervous system and to the hypothalamic pituitary adrenal (HPA) axis. Investigating the cortisol awakening response (CAR) in a subsample of participants, a reduction in the CAR, suggestive of chronic HPA overactivity was correlated with amygdala volumes bilaterally, but more so on the right.

4.4.1. HYPOTHESIS 1: AMYGDALA VOLUME DIFFERENCES EXIST IN MDD AT A NUCLEAR LEVEL BUT NOT AT A WHOLE AMYGDALA LEVEL

The absence of whole amygdala volume differences in MDD (Table 4.7.) in this study is consistent with a majority of studies that have reported no changes in whole amygdala volumes (Zavorotnyy et al., 2018, Sandu et al., 2017, Arnone et al., 2012, Hamilton et al., 2008, Keller et al., 2008). More detailed substructural analysis investigating the constituent parts of the amygdala, i.e. the nine individual amygdalar nuclei and three composite clusters (laterobasal, centromedial and superficial groups), demonstrated that the central and medial nuclei (centromedial group) were altered in state MDD, compared to the control group. The right medial nucleus was found to be larger in MDD ($p=0.002$), with the right and left central nuclei showing distinct trends towards increased ($p=0.069$) and decreased ($p=0.051$) volumes respectively in depression. The right centromedial composite was also relatively increased in volume in the MDD group ($p=0.01$). The potential importance and role of the central and medial nuclei will be expounded in the following sections.

The paralaminar nucleus on the left was also smaller in depression. This somewhat obscure but unique nucleus is grouped with the basolateral substructures. Relatively expanded in humans and non-human primates compared to other species, the paralaminar nucleus expresses high concentrations of receptors for corticotrophin releasing hormone (Sanchez *et al.*, 1999) and GABA-A (Zezula *et al.*, 1988). This makes this area especially responsive to stress related events, and also a site for the action of benzodiazepines, often used in the treatment of anxiety and stress. There are also dense innervations of serotonergic fibres in this small region (Bauman and Amaral, 2005), suggesting this may be a key site where the midbrain monoamine projections interface with and modulate the amygdala. Interestingly, high concentrations of immature cells in the paralaminar nucleus suggest a special mechanism for the nucleus in neuroplasticity (deCampo and Fudge, 2012), an often stress sensitive activity. The statistically significant volume reduction of the paralaminar nucleus found in our MDD patients is particularly intriguing considering these unique affective regulating and affective dependent properties of the region. Interestingly, inputs from the CA1 region (and subiculum) specifically target the ventral basal nucleus and paralaminar nucleus of the amygdala. This is particularly relevant considering the reduced left CA1 volume found in the same MDD cohort (see Chapter 3) and hints at a potential functional interaction between these two areas in MDD.

Overall, the central, medial and paralaminar nuclei differences suggest that substructural analysis of the amygdala may have a greater potential role in the amygdalar study of depression than just whole amygdala volumes on their own. To my knowledge this is the first study to investigate multiple amygdala nuclei or composite nuclei volumes in depression using either an automated or manual segmentation technique.

4.4.2. HYPOTHESIS 2: AMYGDALA VOLUME DIFFERENCES IN MDD ARE PREDOMINATELY LOCATED IN THE CENTROMEDIAL NUCLEI

The centromedial nuclei provide the majority of amygdala output to the paraventricular nucleus of the hypothalamus (Silverman et al., 1981, Sawchenko and Swanson, 1983), increasing downstream cortisol output in a feedforward fashion (Myers et al., 2012). Increases in volume measures in these output nuclei probably, but not definitely, reflect an increased the amygdala drive in stimulating CRH output. The findings in my analysis support this relationship between an increase in the volume of amygdala output nuclei and HPA axis output. The increased centromedial volume found in the MDD cohort is consistent with other evidence linking chronic stress and HPA axis overactivation with depression (O'Keane et al., 2012, Doolin et al., 2017). An important serendipitous finding was that, the right medial and left central nuclei volumes predicted depression with 66% accuracy of ($p=0.002$) across the sample, suggesting a role for these stress-linked output nuclei as potential biomarkers for MDD.

Throughout the analysis, there was a consistent statistical signal from the central and medial nuclei in MDD implying their involvement in the pathology of MDD. This was particularly evident on the right side. Using amygdala composite measures, the right centromedial groups showed an increase on the right ($p=0.01$). Both nuclei are involved in amygdalar output and are also continuous with the bed nuclei of the stria terminalis in what is termed the extended amygdala complex (Heimer, 2003, Alheid, 2003). The extended amygdala is characterised by its abundant connections with regulatory midbrain and endocrine hypothalamic areas (Lebow and Chen, 2016). Accordingly, the centromedial nuclei may act as a potential orchestrator of emotional dysregulation. The right medial nucleus was found to be larger in depression ($p=0.002$). The right central nucleus showed a similar trend towards increased volume ($p=0.095$), whereas the left central nucleus showed a trend towards decreased volume in MDD ($p=0.059$). See Table 4.5., 4.6. and 4.7.

4.4.3. HYPOTHESIS 3: AMYGDALA ASYMMETRY OCCURS IN MDD, REFLECTING THE DIFFERENCE IN EMOTIONAL RESPONSE

Depressed patients showed an increased right-left asymmetry of whole amygdala volumes (R-L delta) (Table 4.8.). The left amygdala has been shown to be involved in the induction of positive emotions through direct stimulation during neurosurgery (Lanteaume et al., 2006). Subjective reward has also demonstrated left amygdalar activation in fMRI (Mormann et al., 2017). In contrast, the right amygdala is more involved in autonomic arousal and negative emotional states (Liu et al., 2015, Lanteaume et al., 2006). In general, the right amygdala is involved in rapid automatic unconscious appraisal of stimuli (with subsequent autonomic arousal), whereas the left evaluates more complex conscious emotional stimuli over a longer latency period (Gläscher and Adolphs, 2003, Bonnet et al., 2015, Sergerie et al., 2008, Diano et al., 2017). Normal amygdala asymmetry is well recognised in humans with the right human amygdala commonly larger than the left in both post-mortem (Murphy et al., 1987) and volumetric MRI studies (Pedraza et al., 2004). This normal right-left amygdala imbalance is amplified in our depressed patients, suggesting that depression may result in a divergent increase in right sided reflexive autonomic emotional arousal along with a corresponding attenuated left sided response to positive emotions and perceptual nuance. The right-left asymmetry for the central nucleus in particular was significantly different in MDD compared to controls (Table 4.8. and Figure 4.6.). The central nucleus was the principal driver of the whole amygdalar right-left difference found in patients. Similarly, the right-left asymmetry for the medial nucleus showed a very strong trend ($p=0.014$) in MDD but did not reach significance following strict correction for multiple comparisons using FDR correction (see Table 4.8. and Figure 4.7.). A schematic diagram showing the whole amygdala asymmetry is shown in Figure 4.8. In this diagram, the amygdalae volumetric differences are exaggerated for effect.

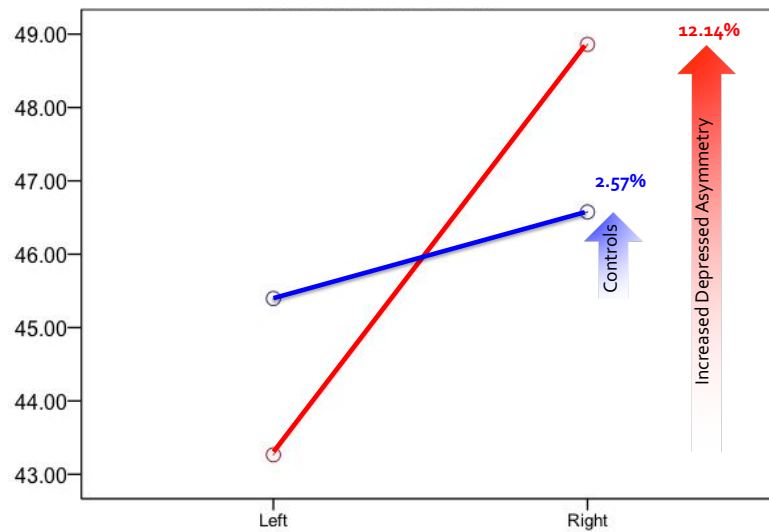


Figure 4.7. Central nucleus asymmetry. Graph showing central nucleus asymmetry for left and right and between depressed and control. Controls are blue and depressed are in red. Note the greater percentage difference between left and right central nuclei (12.14%) in depressed versus controls (2.57%).

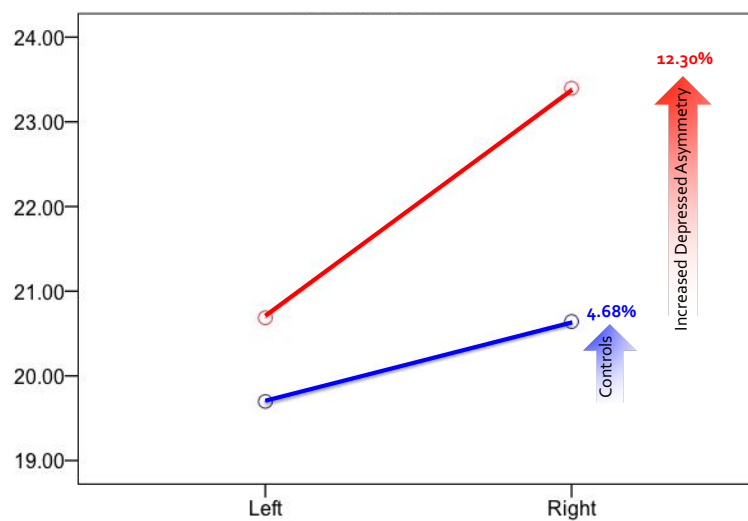


Figure 4.7. Medial nucleus asymmetry. Graph showing medial nucleus asymmetry for left and right and between depressed and control. Controls are blue and depressed are in red. Note the greater percentage difference between left and right medial nuclei (12.30%) in depressed versus controls (4.68%) as in previous figure.

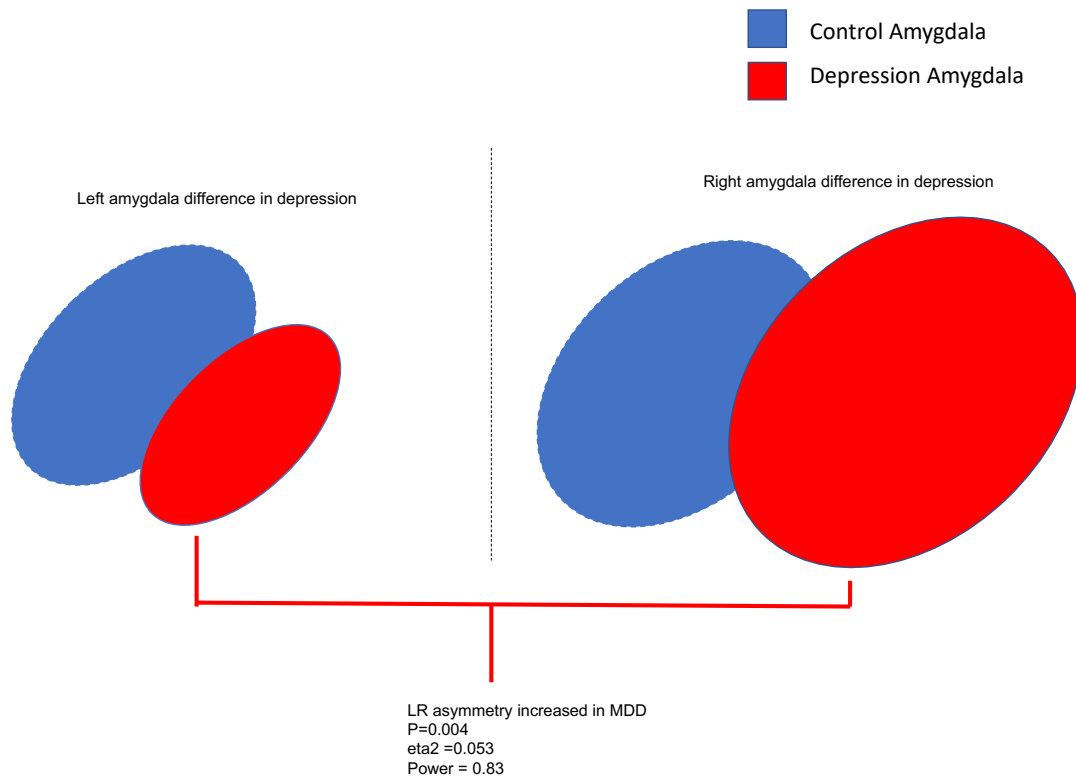


Figure 4.8. Whole amygdala asymmetry. Schematic of whole amygdala asymmetry. Relative sizes are exaggerated for effect. Control amygdala is in blue and depressed amygdala is in red. MDD, major depressive disorder.

As stated above, using binomial hierarchical regression, the right medial and left central nuclei volumes overall predicted a diagnosis of depression with accuracy of 66% ($p=0.002$) across the sample. Interestingly, using linear hierarchical regression, the duration of depression within the MDD group was predicted significantly by right-left volumetric difference (asymmetry) for both central and medial volumes, increasing the accuracy of the overall model from 28% to 35.2% ($p=0.00001$).

4.4.4. *HYPOTHESIS 4: THE CAR, AS A MEASURE OF CHRONIC HPA AXIS OVERACTIVITY, IS NEGATIVELY CORRELATED WITH WHOLE AND NUCLEAR AMYGDALAR VOLUMES IN MDD*

As the centromedial nuclear group consists of the principal output nuclei from the amygdala to the hypothalamus, I investigated measures of HPA axis activity using a previously analysed CAR. The CAR measures the elasticity of the HPA stress axis in response to the natural stress of waking, with decreased CAR reactivity (i.e. a flattened, less buoyant cortisol response to waking) associated with a HPA axis nearing capacity (Farrell et al., 2018). Chronic HPA overactivity as demonstrated by a reduced CAR is commonly found in depression (O'Keane et al., 2012, Juruena et al., 2018, Doolin et al., 2017). Although a relationship between the CAR and hippocampal volumes has been found in MDD (Dedovic et al., 2010), the majority of studies have not shown consistent associations between cortisol measures and hippocampal volumes in the diseased state (Frodl and O'Keane, 2013). There do not appear to be published studies that have investigated the CAR with amygdalar volumes in depression. My findings show that a flattened CAR in MDD is associated with larger amygdala across multiple measures of amygdala volume determination. Substructure analysis revealed negative correlations between the CAR and several nuclei bilaterally in MDD. These negative associations were more extensive on the right. On the right side, a flattened CAR was associated with volume increases across most major nuclei, including basolateral, medial and superficial groups. Conversely on the left, only the basolateral nuclei composite was associated with CAR changes. Interestingly, the centromedial nuclei were only negatively correlated with morning cortisol reactivity on the right side. In animal studies, the central nucleus is involved in modulating HPA responses to systemic or "physical" stress only (Dayas et al., 1999), whereas the basolateral and medial nuclei are activated by both physical and psychogenic stressors (Jones et al., 2011, Myers et al., 2012) (see also Figure 4.2). These findings, at a basic physiological level, provide some evidence that the human amygdala promotes hypothalamic HPA axis drive similar to animals (Giardino *et al.*, 2018). The relationship between increased right amygdalar output nuclei and HPA axis responsivity suggest that amygdalar drive of the HPA axis may be increased in MDD.

More specifically these findings suggest that larger right medial and bilateral basolateral regions are associated with a flattened CAR in MDD, suggestive of a chronic overactivation of the HPA in MDD.

4.4.5. STRENGTHS AND LIMITATIONS

This would seem to be the first time, using published studies as the only indicator, that a comprehensive analysis of amygdala substructure volumes has been undertaken in depression. To my knowledge, it is also the first time that cortisol-awakening responses have been correlated to amygdala volumes. The purpose of this study was to investigate whole and substructural amygdala volumes in depression. Automated segmentation has been argued to have interpretation and validity issues compared to expert manual segmentation (Dill et al., 2015) (see also Chapter 3.4.1). However, considering the vast amounts of data generated in modern neuroimaging studies – for example in this chapter alone 163 subjects yielded almost 2000 individual amygdala substructures - automated segmentation is a far more reliable and viable alternative than manual segmentation for separating the amygdalar nuclei in such large datasets. Only a subset of the total cohort underwent CAR analysis, due to the failure of many participants, especially controls, to engage with the cortisol sample collection. The CAR itself, as a measure of the HPA axis activity, can also be subject to many misconceptions (Fries et al., 2009) and these amygdala-HPA axis findings therefore need to be interpreted with caution. Hypothalamic influence on emotional states is not solely confined to HPA modulation; the ANS also modulates the ‘feelings’ of emotion through changes in sympathetic (arousal – fight or flight) and parasympathetic (relaxation – rest and digest) drive (Beissner et al., 2013, Gallagher and Chiba, 1996). It is likely that the balance of these ANS systems determines subjective affective experiences. ANS function was not measured in this study and we suggest that future neuroimaging studies examining both amygdala measures and ANS output may reveal further information on the functional role of the amygdala substructures in emotional states. Due to the high proportion of total MDD patients prescribed antidepressants (87%), it was not possible to look at the effect of medication on amygdala volumes. This is particularly relevant as antidepressant

medication was the only variable suggested to have effect on whole amygdala volumes in a previous meta-analysis (Hamilton et al., 2008). Sex and age were corrected for throughout the analysis, similar to the hippocampal analysis, so that I was unable to directly assess the effect of these variables in the sample. Also, due to time constraints and the relatively low numbers of completed cortisol samples, I did not investigate first presentation versus recurrent presentation as in the hippocampal analysis in Chapter 3. Future work investigating the longitudinal impact of depression on amygdala volumes will further clarify the mechanism of amygdalar substructural change in MDD.

Finally, without white matter tract analysis of the specific amygdala-hypothalamic connections, the increased amygdala output hypothesis in state MDD cannot be stated with certainty. Correlation does not equal causation. However, an examination of the fibre tracts between the amygdala and the hypothalamus would strengthen this hypothesis. The major white matter tract from the amygdala to the hypothalamus, the stria terminalis is the topic of Chapter 6.

4.5. CONCLUSION

Although the amygdala is a limbic key structure involved in integrating, evaluating and executing emotional states, previous research has failed to find consistent volumetric differences in depressed patients. Using advanced automated amygdala segmentation and novel reassembly of the amygdala nuclei, I have demonstrated increased right-left asymmetry in amygdala volumes with relative larger right to left amygdala volumes in depression. This asymmetry increase (right relative to left), was accounted for mainly by increased right-left asymmetry in the central and medial nuclei, again to the right. These nuclei may also have potential as depression biomarkers as they seem to be highly predictive for both MDD diagnosis and illness

duration. Investigating the HPA stress axis through the CAR in a subset of individuals showed a flattened cortisol response in depressed patients that was strongly correlated with amygdala volume measures, both at a whole amygdalar and nuclei level. These findings are consistent with the known human and animal laterality of amygdalar function and their nuclei and their postulated roles in depression. These findings are also consistent with the hypothesis of an amygdalar driven dysregulated stress response in depression.

CHAPTER 5

THE FORNIX:

HIPPOCAMPAL OUTPUT IN DEPRESSION

"All experience is an arch, to build upon."

Henry Adams

American Historian (1838-1919)

5.1. INTRODUCTION

The fornix (latin; *fornix*, **arch**) is an arched shaped white matter structure linking the hippocampus to the mammillary bodies, hypothalamus, anterior nucleus of the thalamus and septal regions. This structure emerges from the hippocampus along the alveus, which evolves into the fimbriae along the dorsal aspect of the hippocampus (Shah et al., 2012). The fimbriae in turn coalesce into the crura (legs) of the fornix that arch 180 degrees and merge in the midline to form the body of the fornix (Carpenter, 1991). Fibres from the body divert to innervate the anterior thalamus as it passes dorsal to the structure. The body of the fornix then descends, and it becomes the columns of the fornix. As it descends it splits into two separate columns (precommissural and postcommissural) over each side of the anterior commissure. The precommissural columns terminate in the septal and basal forebrain areas whereas the postcommissural columns terminate in the hypothalamus.

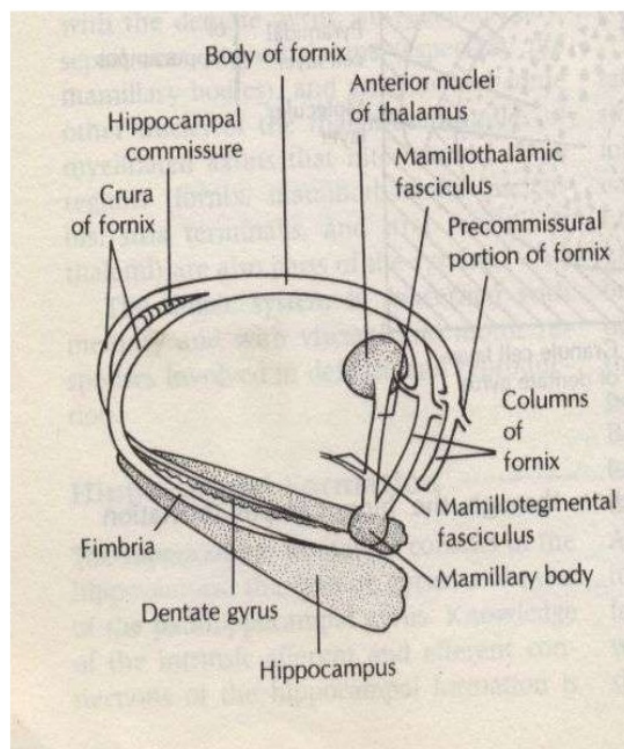


Figure 5.1 The Fornix and its connections. The “columns of the fornix” in the diagram is actually the postcommissural fornix. Taken from Carpenter MB. *Core text of neuroanatomy*. 4th ed. ed. Baltimore: Williams & Wilkins; 1991.

As the principal output pathway of the hippocampus, the fornix is a pathway through which encoded memory from the hippocampus, including spatial information relays to the mammillary bodies and anterior thalamus. Both the mammillary bodies and anterior thalamus (and their connecting tract, the mamillothalamic tract) are important in recollective memory in humans (Vann, 2010). Recollective memory is the type of memory involved when an individual recalls a specific episode from their past experience (Brewer, 1996). Transecting the fornix in rats has shown that the fornix, anterior thalamus and hippocampus are all also important for spatial learning. In particular, the fornix has been suggested to have functionality in resolving problems with spatial and temporal cues (Dumont et al., 2015). Both hippocampal and anterior fornical lesions can greatly impair these so-called biconditional learning tasks that involve simultaneous resolution of both spatial and temporal cues (Albasser et al., 2013, Dumont et al., 2014, Sziklas and Petrides, 2004, Sziklas and Petrides, 2002, Sziklas and Petrides, 1999). However, interestingly, lesions of the fornix are less disruptive than either hippocampal or anterior thalamic lesions on tests of spatial learning and memory (McDonald et al., 1997). This is especially striking considering because lesions of the fornix should theoretically have a greater overall impact, disconnecting the hippocampal formation from multiple sites across the limbic system (Swanson, 1987).

Congenital absence of the fornix in humans is rare (Takahashi et al., 2003), and is associated with failure of development of the septal and other basal forebrain structures. In a case of a recurrent grade III astrocytoma involving infiltration of both fornices, a patient displayed a progressive deterioration in memory that eventually resulted in akinetic mutism (Oberndorfer et al., 2002). Similarly, a post-mortem study of a patient with multiple sclerosis and dementia who had prominent short-term memory impairment and learning impairment showed plaques in both columns of the fornix (Van Paesschen et al., 1997). Wernicke's encephalopathy, a well-described neuropsychiatric condition following thiamine deficiency involves a relatively selective mammillary body degeneration. It presents with the classic clinical triad of confusion (time and place disorientation), ataxia and ophthalmoplegia (paralysis of

certain eye muscles) and is also associated with fornix lesions (Zuccoli and Pipitone, 2009, Weidauer et al., 2003). This may be due axonal loss driven by either retrograde mammillary body degradation or neuroplastic/trophic changes secondary to diminished tract activity.

The fornix has also been implicated in multiple neuropsychiatric diseases and has been proposed as a potential early marker for Alzheimer's disease, multiple sclerosis, schizophrenia and as a possible index for normal ageing (Douet and Chang, 2014). Atrophy of the fornix is well documented in Alzheimer's disease (Callen et al., 2001, Copenhaver et al., 2006). Diffusion tensor imaging (DTI) has also shown reduced fractional anisotropy (FA) of the fornix in Alzheimer's disease including those genetically at risk for dementia (Ringman et al., 2007, Oishi et al., 2012, Mielke et al., 2012). Abnormalities in the fornix have been found in patients with a diagnosis of schizophrenia and are most likely due to degeneration, involving both axonal injury and demyelination, of the fornix (Takei et al., 2008, Fitzsimmons et al., 2009, Fitzsimmons et al., 2014, Davenport et al., 2010, Abdul-Rahman et al., 2011). Changes have also been found in attention deficit hyperactivity disorder (Davenport et al., 2010).

The evidence implicating the fornix in depression, however, is more limited. Considering the close relationship of the fornix with the hippocampus and the abundant evidence implicating the hippocampus in depression (Roddy et al., 2018a, Arnone et al., 2012, MacQueen and Frodl, 2011, Serafini, 2012, Videbech and Ravnkilde, 2004), the fornix may have a potential role in MDD. Despite some non-specific findings little evidence exists that definitively implicates the fornix in depression. Widespread white matter changes, including in the fornix, have been found in MDD patients (Hollocks et al., 2015) and depressive symptoms following brain injury have been associated with global white matter changes, again including the fornix (Raikes et al., 2018). However, some studies have found no changes in the fornix in depression. No differences were found in the structure of the fornix of post-

mortem depressed patients (Brisch et al., 2008, Williams et al., 2015) whereas other white matter tracts showed evidence of degradation. Similarly, a diffusion study of eighty-six patients and sixty-four controls found no differences in white matter integrity of the fornix in MDD, but found differences across multiple limbic white matter tracts (Choi et al., 2016). The closely aligned stria terminalis (see Chapter 6), but not the fornix, was reported to be a predictor of treatment outcomes in a study involving 102 patients with MDD (Korgaonkar et al., 2014). Finally, past childhood adversity in 147 apparently healthy adults was not associated with any changes in the fornix, but was associated with white matter alterations in the corona radiata, corpus callosum, cingulum bundle and uncinate fasciculus (McCarthy-Jones et al., 2018).

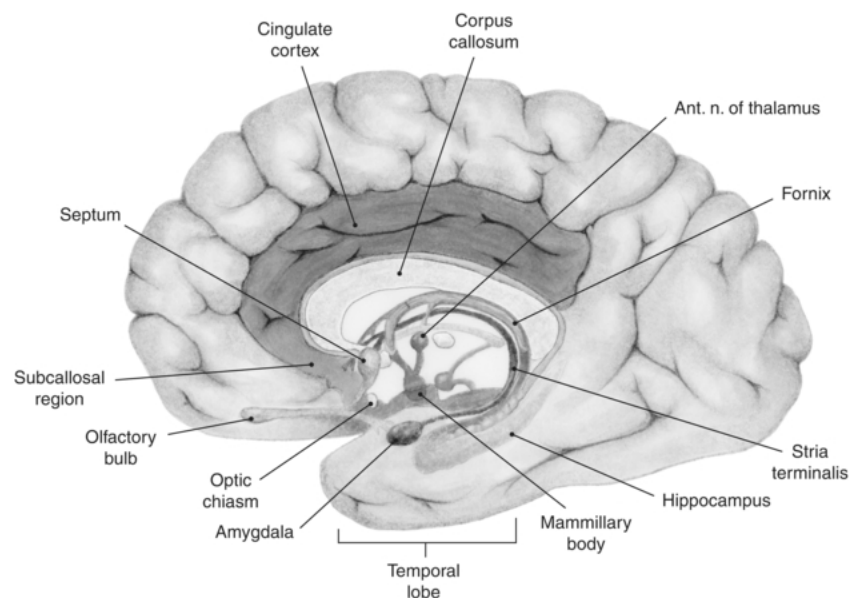


Figure 5.2. The closely aligned pathways of the fornix and stria terminalis. This mid sagittal section of the brain shows how closely aligned the stria terminalis and fornix are along almost all their route. This poses particular problems for diffusion techniques in separating the two tracts. Taken from Carpenter MB. *Core text of neuroanatomy. 4th ed. ed. Baltimore: Williams & Wilkins; 1991.*

Even though the fornix is clearly seen in standard clinical T1 and T2 MRI images of the brain, the fornix is difficult to differentiate from the much smaller and more diffuse stria terminalis. Diffusion weighted imaging in particular has to grapple with this problem. The closeness of the two structures, and relative dominance of the fornix due to increased thickness can cause blending of the fornix and stria terminalis

diffusion streamlines, leading to the appearance of single structure, which I will refer to as the fornix-stria. The closeness of these structures is illustrated in Figure 5.2. Many studies have used diffusion-weighted imaging to investigate the fornix in health and disease (Thomas et al., 2011) but in most cases these studies are actually examining the fornix-stria combination, rather than the fornix alone.

5.1.1. HYPOTHESES

My hypotheses were that

- 1) fornical dimensional differences exist in depression
- 2) fornical whole-tract diffusion metrics differences suggestive of tract pathology exist in depression, and
- 3) fornical metrics differences suggestive of tract pathology exist in depression along only sections of the tract.

5.2. METHODS

The methods used for this study are described in detail in Chapter 2 (sections 2.2., 2.3., 2.4., 2.5., 2.6., 2.7., and 2.9). A brief summary description is given below.

Thirty people with Major Depressive Disorder (MDD) were compared to thirty healthy controls without MDD. All participants completed the MINI (Mini International Neuropsychiatric Interview) for DSM IV (American Psychiatric Association, 1994) and Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960). All patients required a current MINI diagnosis of MDD with a HAM-D greater than 20. Controls were required to have no active or previous MINI diagnosis and a HAM-D less than 8. Only thirty from each of the original MDD and control groups were chosen for investigation using the fornix protocol due to time constraints (each fornix took approximately an hour

or more to clean (see below). Thirty MDD patients were chosen at random, and matched for age and sex with thirty controls

5.2.1. MRI ACQUISITION

All data was acquired on a Philips (Best, Netherlands) Intera Achieva 3.0 Tesla MR system (32-channel head coil) at Trinity College Institute of Neuroscience, Dublin.

T1. 180 axial high-resolution T1-weighted anatomical images were acquired (T1W-IR1150 sequence, TE = 3.8 ms, TR = 8.4 ms, FOV 230 mm, $0.898 \times 0.898 \text{ mm}^2$, in-plane resolution, slice thickness 0.9 mm, flip angle $\alpha = 8^\circ$).

Diffusion Imaging. Whole brain, High Angular Resolution Diffusion Imaging (HARDI) (Jones and Leemans, 2011, Jones et al., 2013b) was acquired using a spin-echo echo-planar imaging (SE-EPI) pulse sequence (TE = 52 ms, TR = 11,260 ms, flip angle $\alpha = 90^\circ$), FOV 224 mm, 60 axial slices, 2mm^3 isotropic voxels, b-value = 1500 s mm^{-2} in 61 non-collinear gradient directions. This scan lasted 16 minutes.

5.2.2. IMAGE PROCESSING

STRUCTURAL

Cortical reconstruction and segmentation was performed using the *FreeSurfer* 6.0 image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>) (Fischl, 2012a, Fischl and Dale, 2000). The technical details of these procedures are described elsewhere (Fischl et al., 2002b, Desikan et al., 2006, Fischl et al., 1999, Fischl et al., 2004, Reuter et al., 2012, Dale et al., 1999). *FreeSurfer* interrogates T1 contrast differences between substructures using previously defined in-vivo and ex-vivo amygdala atlases to determine substructure characteristics. By selecting the 3T MRI flag and multispectral segmentation in *FreeSurfer* the procedure was optimized. The whole amygdala was reconstructed by summing all the amygdala output. Whole amygdala volumes were converted into masks for use in *ExploreDTI*.

DIFFUSION

Diffusion weighted imaging was corrected for signal drift, Gibbs ringing, eddy currents, head movement and processed according to the methods in Chapter 2. Tractography of the whole brain was generated using CSD modelling of all potential deterministic streamlines throughout the entire brain according to the following parameters; seed point resolution was set at 2 x 2 x 2 mm with a step size of 0.5mm, the FOD (Fibre Orientation Distribution) threshold was set to 0.1, and a maximum angle threshold set to 89 degrees. All steps were undertaken using the software *ExploreDTI*.

5.2.3. TRACTOGRAPHY

Both amygdala masks and whole brain tracts were randomized using a random number generator (<https://stattrek.com/statistics/random-number-generator.aspx>) so the individuals doing the manual tractography and fornix/stria extraction and cleanup were blind to the age, sex and group of each subject. This procedure was undertaken by medical students Anna Martin and Tom Drago after an extensive training period. This randomization was undertaken to prevent bias.

A Boolean logic protocol isolated a fornix-stria combination using AND and OR gates within *ExploreDTI* (see Chapter 2.7.) in each of the 60 (30 controls and 30 depressed) randomised subjects. As the amygdala is highly connected with multiple cortical and subcortical areas, this step was undertaken to limit the number of potential amygdalae generated diffusion streamlines to those of the fornix and stria.

The fornix is the primary output from the hippocampus (Thomas et al., 2011). Using the 'Calculate tracts from folder of masks' option both bilateral amygdala volume masks (generated above from *FreeSurfer*) were used as combined NOT gates with the

fornix-stria tracts. This generated a group of streamlines from the bilateral fornix-stria that excluded the stria terminalis bilaterally. Following this, a simple AND gate around the crus of each fornix generated the left and right fornices (See Figure 5.3.). The fornix was defined for the purposes of consistent analysis as from just above the anterior commissure to the mid-section of the crus (where the crus is travelling completely superiorly). The 'tract splitter' tool (Settings→Analysis→Splitter Tool) was used to cut off fornix fibres peripheral to these points and preserve the fibres between.

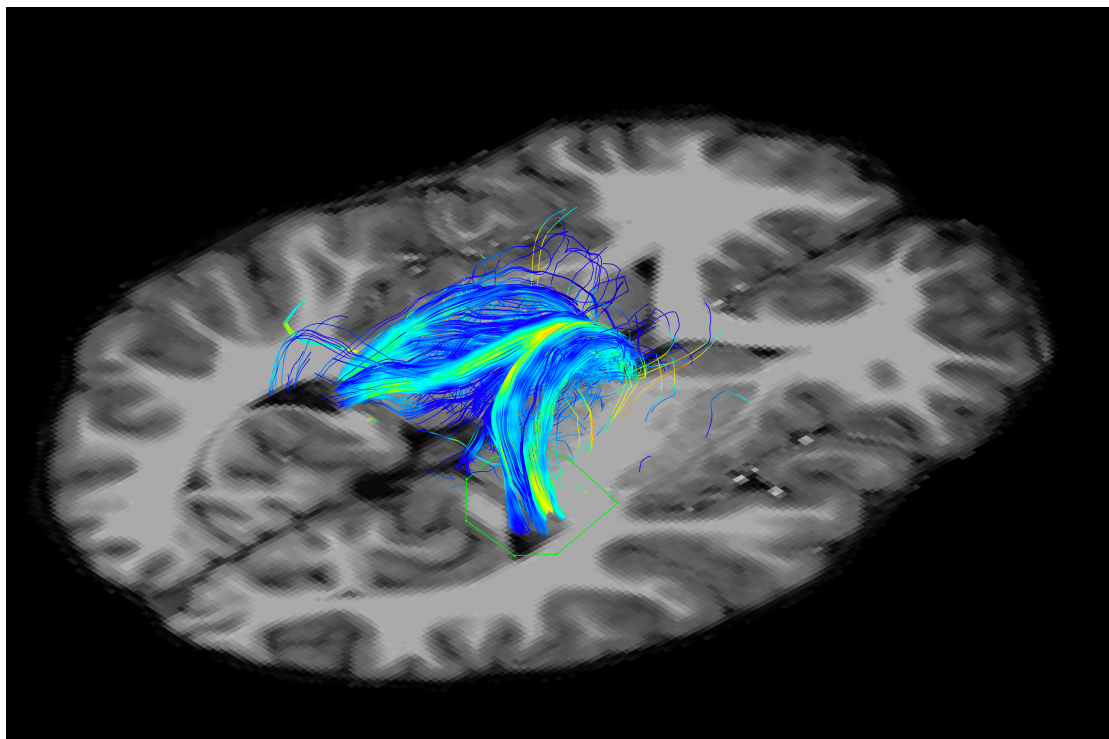


Figure 5.3. AND gate used to separate bilateral fornices. Axial T1 image, showing bilateral fornices with an AND gate around the crus of the right fornix. This logic only reconstructs the left fornix. The right fornix is reconstructed in similar manner.

The next step required that streamlines not associated with the fornix be removed. Cleanup was achieved with two raters (medical students Anurag Nasa and Caoimhe Gaughan) and a neuroanatomist from the Department of Anatomy at Trinity College Dublin after an extensive training period. Examining every subject individually, the raters collectively excluding streamlines not considered fornix fibres using

appropriately placed NOT gates. Criteria for exclusion included streamlines deviating from the known arch and loop shape of the fornix and streamlines consistent with known adjacent tracts. To address potential bias, the neuroanatomist and raters were blind to the identity of each subject. These NOT gates were variable according to each subject and depended on the individual neuroanatomy of the region. However, commonly excluded streamlines included fibres from the hippocampal commissure, lyra, anterior commissure, the median forebrain bundle, stria medullaris and amygdalofugal pathway and various inter-thalamic connections. A second neuroanatomist reviewed a random subsample of 25 tracts from both raters to check face validity of the fornix (looking at start and end points, the arch shape, the tract's relationship to other structures on MRI images (thalamus, anterior/posterior commissures and third ventricle) as well as the overall general impression of the tract. The second neuroanatomist also checked both raters' tracts simultaneously to investigate if they were occupying approximately the same space. This was considered a sufficient subsample for quality control of interrater reliability.

Following cleaning, each fornix tract was saved. Fornix statistics including dimensions (length and volume), standard diffusion measures such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) and Westin diffusion measures [spherical diffusion (CS), linear diffusion (CL) and planar diffusion (CP)] were calculated on each whole-tract using *ExploreDTI's* built in tract statistics plugin (*Plugins* → *Convert* → *Info of tract *.mat file(s) to *.txt*). Along-tract measures were also calculated using *ExploreDTI's* along-tract analysis function. This involved two steps; uniform resampling of the tract into 29 sections and then summarising the metrics. 29 sections were chosen as the average tract length was found to be 58 mm and the diffusion voxel size of 2 mm² (58/2 = 29). Sampling the tract into any more sections than this will not reveal further information. See Chapter 2.7.3. for tract metric equations.

5.2.4. STATISTICAL ANALYSIS

Demographic information was analysed using the statistical analysis program SPSS 24 (<https://www.ibm.com/analytics/us/en/technology/spss/>). Independent t-tests (Student, 1908) were performed to examine between-group differences in age, and HAM-D for controls versus MDD.

TRACT AVERAGED STATISTICS

A series of repeated-measures ANOVA (analysis of variance) controlled for age, sex and eTIV (estimated total intracranial volume) was used with left and right hemisphere diffusion microscopic metrics (FA, MD, AD and RD) and macroscopic tract measures (length, volume and number of streamlines) entered as the within subject factors and group as the between-subject factor. Descriptives for group means, SEM (standard error of the mean) and 95% confidence intervals (CI) were obtained for each metric.

ALONG- TRACT STATISTICAL INVESTIGATIONS

All data was inspected for outliers and extreme values via the *Explore* tool within SPSS. Multivariate ANCOVA (analysis of covariance) for each diffusion metric (FA, MD, AD and RD) was used to investigate the variation of each metric over the length of the tract of interest. Between-group p-statistics for each metric were extracted for each section. Multiple comparison correction (MCC) was performed using False Discovery Rate (FDR) correction (Benjamini, 2010). To guard against the known negative effect of outlier and extreme outlier values on multivariate statistical tests, all outliers were 'winsorized' (Hastings et al., 1947), which essentially limits the outlier points to the calculated upper and lower 95% CI boundaries. This also ensured additional statistical power, as missing data did not factor in the subsequent "cleaned" data. Age, sex and eTIV was controlled for throughout the analyses.

5.3. RESULTS

DEMOGRAPHICS

See 5.1. Thirty patients and thirty controls were analysed for this part of the study. Fifteen males and fifteen females with good quality diffusion imaging were chosen otherwise at random in each group.

No significant between-group differences between controls and depressed patients were found for age, sex or handedness. The average duration of depression in this subsample was 17 months. The age, HAM-D scores, handedness and duration of depression were all representative of the full (80/83) sample (see Chapter 3, Table 3.3). All thirty patients were taking antidepressants at the time of scanning, compared with 87% of the full depressed group.

	Group		Con v Dep
	Con N=30	Dep N=30	p-value
Age: Mean(SEM)	29.0 (1.4)	31.2 (1.8)	0.13
Range (years)	16-64	17-64	-----
Male/Female (%M)	15/15, 50%	15/15, 50%	X ² : 1
Handedness (R/L)	13/2	14/1	NS
HAMD* (SEM)	1.3(0.2)	25.2(1.4)	5.10E-75
MDD months**	-----	17(6)	-----

Table 5.1. Demographic Data. Con, controls; Dep, depressed; HAM-D, Hamilton Depression scale; M, male; MDD, major depressive disorder; R/L, right or left handed; SEM, standard error of the mean.

5.3.1. TRACT VOLUMETRICS

Anatomical macroscopic tract comparisons revealed no significant main effect for tract length, volume or number of streamlines. Similarly, no between group differences were identified in either length volume or streamlines (See Table 5.2.).

									p-values		
		Left			Right			Within-subject/Interaction	ANCOVA		
	Group	Mean	SEM	95% CI	Mean	SEM	95% CI	Main effect F(dof), p: F(dof), p	ConvDep	Left	Right
Volume	Cons	2631.12	124	2330.72-2905.07	2803.71	105	2527.13-3086.6	1.71(1), 0.20: 0.21(1), 0.65	0.297	0.529	0.216
	Dep	2517.21	124	2311.26-2749.61	2613	105	2396.69-2813.77				
Length	Cons	57.41	0.524	55.43-58.25	57.39	0.871	55.63-58.99	0.583(1), 0.449: 0.041(1), 0.84	0.932	0.798	0.969
	Dep	57.6	0.524	55.29-58.77	57.34	0.871	55.57-59.28				
Lines	Cons	315.06	25.25	261.57-361.36	319.05	18.57	274.05-368.61	0.584(1), 0.448: 0.577(1), 0.451	0.806	0.862	0.465
	Dep	321.44	25.25	271.80-378.27	299.28	18.57	264.14-329.87				

Table 5.2. Fornix volumetrics. Volume, length and number of streamlines of the fornix. ANCOVA, analysis of covariance; CL, linear diffusion coefficient; Cons, controls; Cp, planar diffusion coefficient; Cs, spherical planar coefficient; Dep, depression; dof; degrees of freedom; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; SEM, standard error of the mean.

5.3.2. WHOLE-TRACT DIFFUSION METRICS

Independent repeated measures ANOVA, correcting for age, sex and eTIV, identified significant within-subject main effect for FA ($F[7.68 \text{ (dof1)}]$, $p=0.008$). indicating the presence of lateralized difference for FA, to the right side, with depressed patients showing reduced FA relative to controls. Significant interaction effects were identified for MD, ($F[4.467 \text{ (dof1)}]$, $p=0.036$) and AD ($F[10.78 \text{ (dof1)}]$, $p=0.002$) indicating a complex relationship between group and lateralized fornix diffusion measures with depressed patients showing greater right fornix MD and AD relative to controls. Between-group comparisons failed to identify any significant bilateral total differences for any DTI metric, however AD was decreased on the right side in MDD. See Table 5.3.

5.3.3. ALONG-TRACT DIFFUSION METRICS

ANCOVA for along-tract metrics showed significant differences in only one section on the left side between MDD and controls, after FDR correction, showing an FA decrease in MDD. On the left AD was significantly decreased in MDD across six continuous sections in the anterior section of the tract. See Table 5.4

		Left			Right			Within-Subject/ Interaction Effect		ANCOVA	
DTI Metric	Group	Mean	SEM	95% CI	Mean	SEM	95% CI	Main Effect F(dof), p : Interaction F(dof), p	Between groups	Left (p, partial eta)	Right (p, partial eta)
FA	Cons	0.436	0.007	0.4212-0.4543	0.412	0.006	0.3991-0.4224	7.68(1), 0.008 : 2.795(1), 0.1	0.4	0.125, 0.42	0.702, 0.003
	Dep	0.42	0.007	0.4103-0.4391	0.415	0.006	0.4017-0.4304				
MD	Cons	0.0012614	0.0000184	0.0012-0.0013	0.00120496	0.000021	0.0012-0.0013	1.36(1), 0.25: 4.647(1), 0.036	0.233	0.972, 0.0005	0.089, 0.052
	Dep	0.0012623	0.0000184	0.0012-0.0013	0.0013021	0.000021	0.0012-0.0013				
AD	Cons	0.0019158	0.0000238	0.0018-0.0019	0.001851	0.000026	0.0017-0.0019	1.95(1), 0.17: 10.775(1), 0.002	0.394	0.577, 0.006	0.045, 0.071
	Dep	0.0018964	0.0000238	0.0018-0.0019	0.0019265	0.000026	0.0018-0.0019				
RD	Cons	0.0009241	0.00002	0.0008-0.0009	0.0009111	0.00002	0.0009-0.0010	1.31(1), 0.26: 1.64(1), 0.206	0.167	0.551, 0.007	0.067, 0.060
	Dep	0.0009324	0.00002	0.0009-0.0010	0.0009471	0.00002	0.0009-0.0010				
Westin											
CL	Cons	0.469	0.007	0.4544-0.4869	0.443	0.006	0.4314-0.4541	7.64(1), 0.008: 2.936(1), 0.092	0.613	0.184, 0.032	0.447, 0.011
	Dep	0.455	0.007	0.4447-0.4742	0.449	0.006	0.4365-0.4564				
Cp	Cons	0.07721	0.002	0.0742-0.0805	0.08249	0.0021	0.0783-0.0858	1.55(1), 0.22: 2.53(1), 0.12	0.424	0.695, 0.003	0.124, 0.042
	Dep	0.0783	0.002	0.0737-0.0825	0.07775	0.0021	0.0737-0.0825				
Cs	Cons	0.461	0.006	0.4373-0.4665	0.47	0.006	0.4644-0.4858	7.72(1), 0.007: 1.77(1), 0.19	0.395	0.167, 0.035	0.92, 0.0005
	Dep	0.478	0.006	0.4497-0.4749	0.48	0.006	0.4575-0.4849				

Table 5.3. Fornix whole-tract diffusion metrics. AD shows significance ($p=0.45$) for difference between groups for the left fornix only. No other metrics show significance, although RD and MD show trend levels. AD; axial diffusivity; ANCOVA, analysis of covariance; CL, linear diffusion coefficient; Cons, controls; Cp, planar diffusion coefficient; Cs, spherical planar coefficient; Dep, depression; dof, degrees of freedom; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; SEM, standard error of the me

		Left					Right		
p-value (Con v Dep)									
Section	FA	MD	AD	RD	Section	FA	MD	AD	RD
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29					1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29				
	0.801	0.32	0.825	0.667		0.96	0.305	0.23400	0.035
	0.798	0.857	0.303	0.684		0.967	0.276	0.17700	0.436
	0.851	0.6	0.157	0.353		0.579	0.357	0.13300	0.39
	0.77	0.494	0.328	0.648		0.595	0.312	0.17700	0.15
	0.665	0.486	0.12	0.128		0.454	0.182	0.10600	0.14
	0.696	0.144	0.168	0.9		0.968	0.534	0.32000	0.592
	0.83	0.382	0.132	0.602		0.875	0.653	0.44800	0.785
	0.956	0.247	0.191	0.334		0.701	0.315	0.20200	0.685
	0.516	0.242	0.35	0.63		0.903	0.455	0.48900	0.585
	0.389	0.377	0.351	0.824		0.83	0.462	0.29400	0.536
	0.148	0.633	0.393	0.847		0.646	0.375	0.32600	0.426
	0.034	0.872	0.504	0.641		0.702	0.365	0.34800	0.4
	0.019	0.739	0.336	0.322		0.685	0.271	0.28200	0.285
	0.004	0.723	0.349	0.215		0.223	0.161	0.26600	0.133
	0.006	0.647	0.581	0.212		0.354	0.143	0.19200	0.141
	0.021	0.483	0.301	0.33		0.166	0.105	0.09500	0.139
	0.028	0.787	0.241	0.434		0.957	0.039	0.05300	0.066
	0.049	0.948	0.192	0.368		0.394	0.007	0.01700	0.14
	0.132	0.914	0.296	0.242		0.303	0.019	0.00900	0.068
	0.134	0.971	0.739	0.106		0.209	0.059	0.00200	0.267
	0.082	0.635	0.876	0.052		0.269	0.027	0.00030	0.17
	0.264	0.222	0.571	0.09		0.47	0.005	0.00300	0.04
	0.331	0.191	0.158	0.032		0.088	0.02	0.00100	0.155
	0.175	0.025	0.824	0.232		0.088	0.179	0.00800	0.308
	0.068	0.292	0.279	0.217		0.029	0.16	0.03600	0.392
	0.096	0.222	0.25	0.102		0.257	0.175	0.01500	0.371
	0.034	0.057	0.171	0.049		0.392	0.551	0.21100	0.725
	0.001	0.076	0.259	0.063		0.655	0.766	0.83300	0.607
0.274	0.076	0.312	0.13	0.036	0.234	0.43600	0.193		
Tract Average	0.053	0.278	0.557	0.623	Tract Average	0.149	0.033	0.01900	0.069
FDR	0.001	0	0	0		0	0	0.00900	0

Table 5.4. Fornix along-tract diffusion metrics. Only AD on the right survives strict FDR correction (highlighted in red). Bold indicates significance without FDR correction ($p < 0.05$). Only one section survives FDR on the left. AD; axial diffusivity; CI, confidence interval; Con, controls; Dep, depression; FA, fractional anisotropy; FDR, false discovery rate; MD, mean diffusivity; RD, radial diffusivity.

5.4. DISCUSSION

No differences were found in the numbers of streamlines, lengths or volumes of the fornix between controls and depressed patients. In terms of diffusion metrics for the whole fornix measure, AD was significantly decreased ($p=0.045$) in patients on the right side with a corresponding trend for RD increase ($p=0.067$). Further investigating this difference using along-tract metrics revealed that this was strongly driven by decreased AD in depressed patients along the columns of the right fornix.

5.4.1. *HYPOTHESIS 1: FORNICAL DIMENSIONAL DIFFERENCES EXIST IN DEPRESSION*

No differences were found in either volumes ($p=0.297$), tract length ($p=0.932$) or number of streamlines ($p=0.806$) between depressed patients and controls. The null hypothesis failed to be rejected in this case.

As this is the first study to investigate the fornix independent from the stria terminalis using this technique, I am unable to compare my results to other studies. Volumes and lengths of the fornix have not been reported previously. Also, I selected my fornix length as the distance between the anterior commissure and the mid-crus point. This was chosen for consistency reasons. The initial hippocampal part of the fornix is an emergent structure that gradually coalesces into the fornix from alveus to fimbriae to definite crus. I demarcated from the crus onwards as the most coherent part of the fornix (for metric comparison reasons) and chose this point as the starting point of the fornix. This has inherent limitations; the main one being that the fornices generated as part of this study are shorter than the actual length and this measure will fail to pick up on any other discrepancies between MDD and control groups that may be present in other parts of the fornix, particularly the fimbriae regions. The key advantage of this measure is that it is comparable across subjects. However, the similarity of volume, length and streamline data between MDD and controls is reassuring as it shows that systematic bias when generating the fornix was unlikely.

5.4.2. HYPOTHESIS 2: FORNICAL WHOLE-TRACT DIFFUSION METRICS DIFFERENCES SUGGESTIVE OF TRACT PATHOLOGY EXIST IN DEPRESSION

See Table 5.3. An overall effect for FA was found between left and right hemispheres, but this did not translate to MDD versus controls. An interaction effect for MD and AD was found, suggesting that these metrics and hemisphere and group all act together in a combined way. These were driven by right-sided differences. Examining diffusion metrics for the entire length of the fornix did not reveal any differences in FA, MD, RD and AD between depressed and controls, bilaterally. Examining the differences between controls and depressed for the left and right separately revealed decreased AD measures in MDD in the right fornix ($p=0.045$), with a trend for RD increase ($p=0.067$) and a weaker trend for MD increase ($p=0.089$) in MDD patients.

AD (axial diffusivity) is a measure of diffusion parallel to the principal eigenvector (λ_1) (see Chapter 2.7 for equations) and is considered a proxy measure of axonal damage or injury (Alexander et al., 2007). Diffusion metrics are sensitive to several tissue characteristics (e.g. myelination, axon diameter, fibre density, fibre organization) and need to be interpreted carefully (Mori and Aggarwal, 2014). However, AD is considered to be an estimate of axonal integrity. As the right fornix connects the right hippocampus to the hypothalamus and basal forebrain regions, a decreased AD could reflect reduced integrity of the connections between the right hippocampus and its main target structures.

Decreased AD of the fornix has been found in some studies where MDD and depressive symptoms were examined. In a study of late life depression, decreased AD in the fornix was associated with mild cognitive impairment (Li et al., 2014), and AD decreases of the fornix were also found in Alzheimer's patients with depression, compared to controls or Alzheimer's patients without depression (Zhu et al., 2016). In a study of sleep disturbance, depressive symptoms were associated with decreased AD throughout the limbic system, including the fornix (Raikes et al., 2018). In another

study AD in the fornix and other tracts was found to reduce with age but was unchanged in MDD and those who had experienced childhood adversity (Ugwu et al., 2015) .

The one study that that independently studied both the fornix and stria terminalis, did not investigate AD or RD, instead only reporting FA (Korgaonkar et al., 2014). Similarly, FA, but not RD was reported in many other diffusion studies investigating the fornix in MDD (Bracht et al., 2015, Hollocks et al., 2015, Geng et al., 2016, McCarthy-Jones et al., 2018, Hermens et al., 2018, Duffy et al., 2014, Hoogenboom et al., 2014, Frodl et al., 2012, Malykhin et al., 2008). FA differences in MDD were reported in some of the studies.

5.4.3. HYPOTHESIS 2: FORNICAL METRICS DIFFERENCES SUGGESTIVE OF TRACT PATHOLOGY EXIST IN DEPRESSION ALONG ONLY SECTIONS OF THE TRACT

Along-tract analysis allows deeper exploration of diffusion differences. By dividing each fornix into 29 equal sections (See 5.2. Methods, above), I was able to investigate the four diffusion metrics in each subsection. Section 1 (S1) was the start of the crus of the fornix and section 29 (S29) was just above the anterior commissure (Table 5.4). See Figure 5.4 for diagrammatic representation.

The left fornix in depression showed a difference, following multiple correlation correction with FDR, only in one metric at one section (decreased FA at S28),. A difference in a single section out of twenty-nine is unlikely to be clinical significance. The AD differences found in the whole-tract analysis of the right fornix were found to be sequestered entirely in the anterior part of the tract. S17 to S26 showed decreased AD relative to controls, with S19 to 24 showing true significance following multiple correlation correction with FDR. This corresponds to the anterior part of the body and differences were mostly found in the columns of the fornix on the right (see Table 5.4.). The trend level RD differences in the whole right fornix did not translate into

any localised differences with along-tract analysis. Similar sections (S17-23) showed some differences, but these failed to reach significance following FDR.

It is likely that these differences in the right anterior body and column occur along the fornix distal to where fibres leave the body of the fornix to innervate the anterior thalamus. If this is the case, then the direct hippocampus → anterior thalamus circuit may be unaffected in MDD. The indirect hippocampus → mammillary bodies → anterior thalamus circuit could still be impacted however, by the poorer neuronal integrity further along in the columns. This could contribute to the memory dysfunction experienced by MDD patients, and moves the investigation of cognitive difficulties in depression beyond the hippocampus. Dividing the fornix into pre- and postcommissural components (see Section 5.4.4 below) may reveal if the indirect anterior thalamic connection is affected in depression. Further neuroanatomical study is needed to precisely define where along the body the anterior thalamic branch exits.

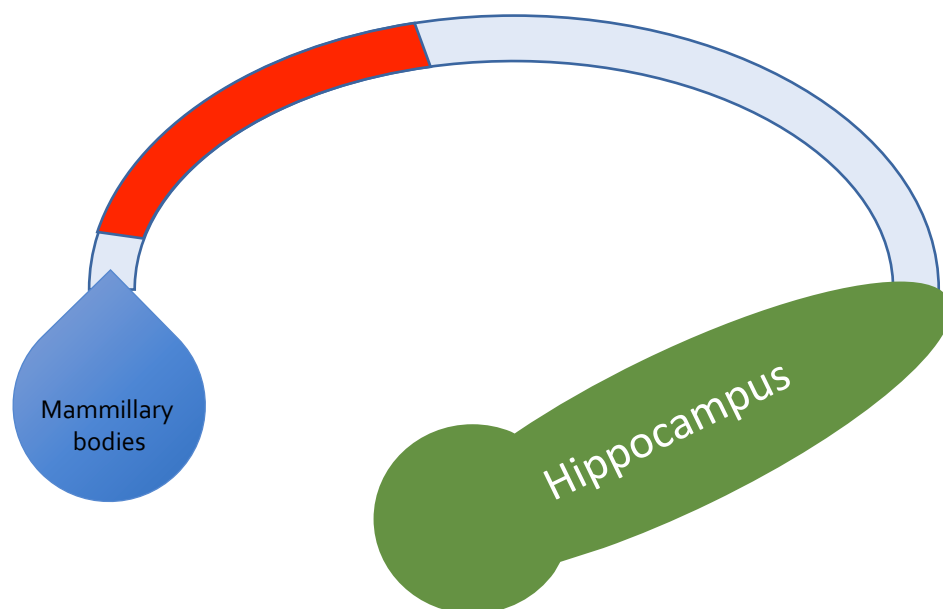


Figure 5.4. Schematic showing fornix and affected tract portions. The red section (anterior third or so) shows the portion of the tract showing decreased AD. This corresponds to the anterior portion of the body and most of the columns of the fornix. AD, axial diffusivity.

Along-tract analysis is a relatively new technique (Colby et al., 2012) and only one published study has used it to survey the fornix. This study examined the fornix in patients with schizophrenia and found FA differences in the fimbriae but not the body of the fornix (Goghari et al., 2014). No AD differences were found between schizophrenia patients and controls. My study did not exam the fimbriae of the fornix. Another as yet unpublished study, which I was involved in (O'Hanlon et al. unpublished), found FA differences in youth with psychotic experiences within the left fornix using along-tract analysis. Again, no difference in AD was found. To date no published stud, to the best of my knowledge has examined the fornix using along-tract analysis in MDD.

Although whole-tract analysis of this cohort revealed AD differences between controls and MDD patients, the significance was relatively weak ($p=0.045$). It is interesting that the along-tract analysis uncovered a localised portion of the fornix on one side with decreased AD at the anterior end of in which particularly significant differences clustered (average p -value in the affected portion = 0.003). This concentrated difference in AD (and RD) is 'diluted' by large portions across the rest of the fornix showing no differences. It is entirely possibly that previous studies failing to identify changes in the fornix in MDD may be masking localised changes such as this.

5.4.4. STRENGTHS AND LIMITATIONS

This is the first study to consistently separate the fornix from the stria terminalis from whole brain tracts using deterministic tractography. Deterministic tractography is an experimental method that is subject to some technical challenges (Jones, 2008). There are known issues involving curving and kissing tracts (see Chapter 2.6.2.). The fornix is highly curved and turns 180° in a relatively tight space. This was addressed by reconstructing the fornix using high angle (89°), small step-size (0.5 mm) whole

brain tracts. This tract lies deep within the centre of the brain, surrounded by and in direct contact with other white matter bundles including the stria terminalis, stria medullaris, and various thalamic connections. CSD was chosen as the most appropriate tractography technique due to its superior ability at detecting complex crossing and kissing fibres in a recent analysis of fibres estimation approaches (Wilkins et al., 2015). As with all diffusion-weighted reconstruction, the generated fornix only describes the diffusivity in the region of interest and it is assumed that this corresponds to an actual tract. A subsample of images (50%) was presented to a second neuroanatomist to check and confirm the face validity of the results. As part of my post-PhD work, I have already started to liaise with the Anatomy Department in both TCD and UCD to measure the fornix (and other tracts) dimensions. This will allow validation of these diffusion techniques. However, the similarity of volume, length and streamline data between MDD and controls is reassuring as it shows that systematic bias when generating the fornix was unlikely.

An experienced neuroanatomist with expertise of the region also aided during cleanup. This represented the most valid approach for removing extraneous streamlines. As the protocol was designed to encompass as much of the fornix as possible in a consistent manner, the initial gates were overly broad. This resulted in a large amount of cleanup. The average time of cleanup (both rater and neuroanatomist working together) was estimated at about 20 minutes per subject. It is appreciated that this approach may not be available or practical for some research groups. However, with adequate neuroanatomical training, this limitation may be overcome, and a researcher/technician experienced with *ExploreDTI* and the complex regional anatomy could single-handedly undertake the entire process.

One particular limitation of my approach is that the posterior portion of the fornix was clipped from the crus downwards. This was done to ensure consistent anatomical landmarks (i.e. the anterior commissure and crus) when segmenting out the fornix. This is due to the relative disorganisation and incoherence of the diffusivity

streamlines of the fimbriae as they emerge from the hippocampus. This protocol was also chosen to be comparable to the protocol for the stria terminalis. Therefore, the portion of the tract along the dorsal aspect of the posterior hippocampus to the crus (i.e. the fimbriae) is not considered in this thesis. The generated tract considered here is the dorsal fornix only. As FA differences have been found in the fimbriae, but not the body of the fornix in schizophrenia (Goghari et al., 2014), this region should not be ignored. Post-PhD research will investigate the fimbriae areas.

During the analysis it was noted that the fornices generated using this method had clear pre- and postcommissural components as it diverged across the anterior commissure (See Figure 5.5). These are known to connect the hippocampus to the septal areas/basal forebrain and mammillary bodies/hypothalamus respectively (Carpenter, 1991). It is possible that extracting each of these components of the fornix independently could yield further information about circuit-specific differences of the fornix in MDD (Jang and Yeo, 2017, Yeo et al., 2013). Potentially, changes in the precommissural fornix, associated with hippocampus → septal/basal forebrain connections may reveal associations with the MDD symptoms of amotivation, lethargy, anhedonia, memory formation and retrieval and cognition, relating to the functions of these areas. Similarly, changes in the postcommissural fornix, associated with hippocampus → mammillary body/hypothalamic connections may be associated with difficulties in memory formation, vegetative disturbances and increased stress reactivity, relating to the functions of these areas. Considering the AD reductions isolated to the column of the right fornix found in this study in MDD, separating out the components that innervate either the motivation or memory centres may yield fruitful further information concerning these circuits in depression. Post-PhD work on this has already commenced, isolating each of the two 'subtracts' of the fornix and relating each of these individual subtracts to symptoms from the HAM-D assessments.

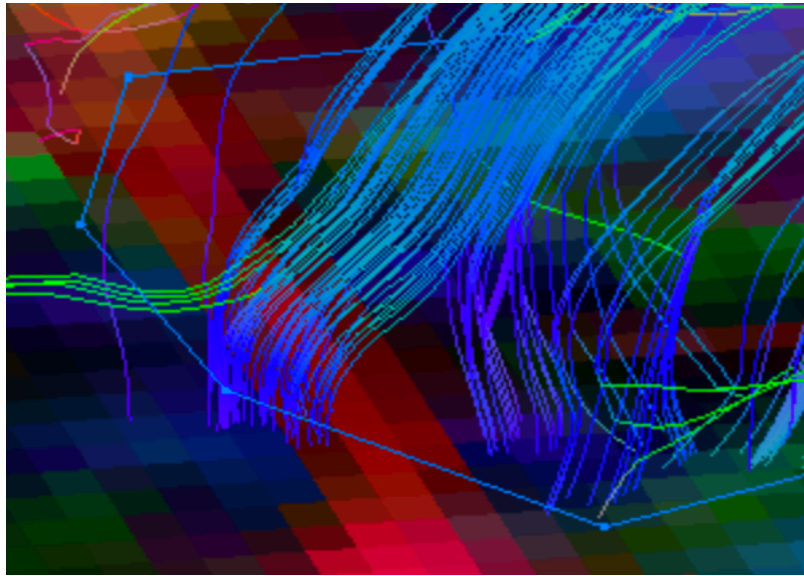


Figure 5.5. Magnified view of pre and post commissural fornix. Shown are the pre and post commissural fornix of a subject. The commissure is the continuous red line. The blue gate (SEED gate) can be clearly seen surrounding the fornix columns.

5.5. CONCLUSION

The fornix as the principal output from the hippocampus may play a role in depression. In this chapter I have demonstrated how to generate a pure fornix using a novel technique, separating it from the adjacent stria terminalis and how this technique may be used to investigate differences in MDD. No differences in the dimensions of the fornix were found in depression. Whole-tract diffusion metrics showed decreased AD on the right side in depression. Using along-tract analysis for deeper exploration of the sections of the tract showed that this whole-tract difference was driven by localised decreased AD in the right column. These focal decreases in AD in the fornix are suggestive of abnormal neuronal integrity in the columns of the right fornix in MDD.

CHAPTER 6

THE STRIA TERMINALIS:

AMYGDALA OUTPUT IN DEPRESSION

"Every time I look at a zebra,
I can't figure out whether it's black with white stripes or white with black stripes,
And that frustrates me."

Jodi Picoult,
House Rules, 2010

6.1. INTRODUCTION

The stria terminalis (latin; *stria*, **stripe**; *terminalis*, **terminal**) is an arched shaped white matter structure linking the amygdala to the hypothalamus and septal regions. This structure emerges from the centromedial nuclei of the amygdala, even though it should be noted that it also contains fibres that arise directly from the laterobasal nuclei (Cowan et al., 1965, Cassell et al., 1999, Alheid, 2003). After travelling along the dorsal aspect of the hippocampus, the fibres coalesce into the crura of the stria that arch 180 degrees over the thalamus, similar to the fornix. The stria terminalis travels rostrally, adjacent and slightly lateral to the body of the fornix, before dipping caudally with branches each side of the commissure, again similar to the fornix. Along its course, the stria terminalis is accompanied by the superior thalamostriate vein (or terminal vein), which lies underneath (Carpenter, 1991) (Figure 6.1). The precommissural columns terminate in the septal and basal forebrain areas whereas the postcommissural columns terminate in the hypothalamus, once again similar to the fornix.

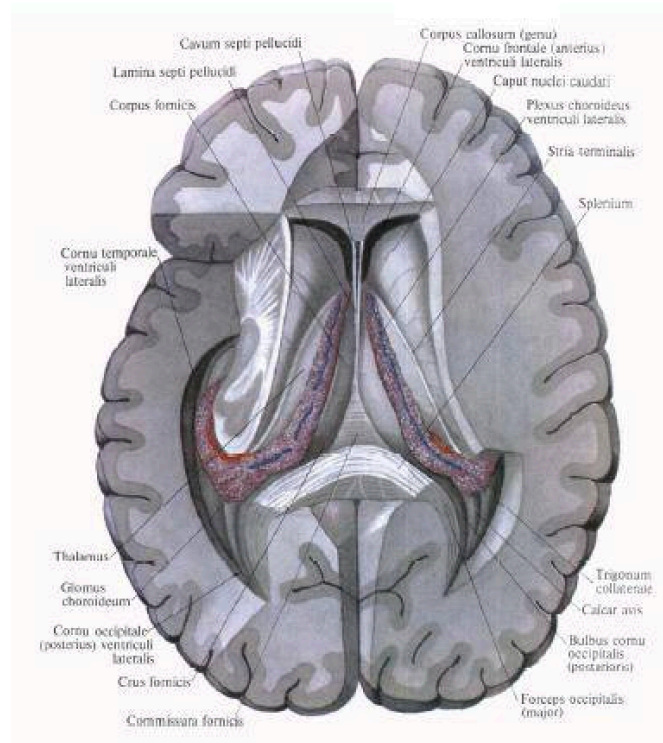


Figure 6.1. The stria terminalis and fornix. Superior axial drawing of the brain, with the brain sliced in the axial plane to show the stria terminalis and fornix as they travel superior to the thalami. Note the blue coloured thalamostriate vein and overlying choroid plexus (purple/red). Taken from Carpenter MB. *Core text of neuroanatomy*. 4th ed. ed. Baltimore: Williams & Wilkins; 1991.

Despite its similarities in shape, and some of the termination points, the stria terminalis is different from the fornix in the following ways;

- a) The stria terminalis originates in the amygdala as opposed to the hippocampus.
- b) The stria terminalis does not innervate the anterior thalamic nuclei.
- c) The stria terminalis predominantly innervates the more medial nuclei of the hypothalamus, as opposed to the more lateral nuclei and mamillary bodies from the fornix.
- d) The stria terminalis is not just a primarily connective white matter structure and contains diffusive processing centres throughout the anterior section.
- e) The human stria terminalis is overall much less distinctive and defined than the fornix.

Concerning point d) above, the bed nucleus of the stria terminalis (BNST) is located at the caudal extremity of the stria terminalis and consists of a cluster of twelve to eighteen nuclei surrounding the caudal part of the anterior commissure (Dumont, 2009). The BNST is a small but highly integrative complex structure that extends from the nucleus accumbens region to the amygdala along the stria terminalis. It is a processing centre in its own right, receiving inputs from the amygdala, which are relayed to the hypothalamus and brainstem nuclei (Dumont, 2009, Weller and Smith, 1982). However, the BNST, stria terminalis and output nuclei of the amygdala (central and medial) are considered part of an extended amygdala complex, an all-inclusive term describing a distributed hub integrating and relaying autonomic, neuroendocrine and behavioural aspects of brain function (Heimer et al., 1999, Lebow and Chen, 2016).

As the principal output tract from the amygdala to the hypothalamus (the other being the amygdalofugal pathway), the stria terminalis connections from the central and medial nuclei of the amygdala to the paraventricular nucleus of the hypothalamus along the stria terminalis function to drive hypothalamic pituitary adrenal (HPA) axis

output in response to stress. This is the main mechanism by which the amygdala transduces the stress response through increasing downstream cortisol output. Possible amygdalar drive of the HPA axis is potentially germane to the HPA axis overdrive that occurs in MDD.

Imaging the structure of the stria terminalis is difficult. Lying close to the fornix, it has a smaller diameter and is dominated by this much larger white matter structure. It is also relatively diffuse compared to the fornix and other white matter tracts. Most studies that measure the fornix either through probabilistic or deterministic diffusion techniques, do not mention the removal of the stria terminalis and can be considered to include this structure. Three studies have previously used diffusion imaging in an attempt to visualise this white matter tract (Kamali et al., 2015b, Kruger et al., 2015, Korgaonkar et al., 2014). One study demonstrated precommissural connectivity to the septal areas and basal forebrain and postcommissural connectivity to the hypothalamus similar to that of the fornix (Kamali et al., 2015b). Another study showed diffuse (but non-specific) connectivity of the BNST across the hypothalamic and frontal areas (Kruger et al., 2015). Both these techniques used probabilistic tractography. While useful for exploratory studies, this technique is not anatomically driven and thus not suitable for accurate stereotactic localisation of the tract. It is also not as useful as deterministic tractography for precise regional quantification of diffusion metrics.

THE STRIA TERMINALIS IN DEPRESSION

Due to the inherent difficulties in imaging the stria terminalis, there has only been one diffusion study showing in-vivo differences in humans in depression. In 102 patients with MDD, integrity of the stria terminalis, but not of the fornix was associated with treatment outcomes in patients (Korgaonkar et al., 2014). Those patients with a lower fractional anisotropy (FA) were more likely to achieve remission. However, in animal studies, the stria terminalis, and more specifically the grey matter BNST, has been implicated in augmenting the stress response (LeDoux et al., 1988,

Greenwood et al., 2005, Muigg et al., 2007, Luyck et al., 2017) and in the potential pathophysiology of depression (Stone et al., 2006, Crestani et al., 2010). Activation of the stress response through stria terminalis stimulation in animal models can also be attenuated following treatment with anti-depressant medications (Muigg et al., 2007).

Deep brain stimulation of the stria terminalis, around the regions of the BNST, has been recently reported to improve MDD symptoms. In one pilot study consisting of five patients with severe treatment resistant depression, 2/5 achieved sustained remission and a further 2/5 achieved notable therapeutic benefits (Fitzgerald et al., 2018). A published case study involving a single MDD patient with comorbid anorexia nervosa reported improvement following stimulation of both the medial forebrain bundle (a closely aligned pathway to the stria terminalis, involved in reward) and the stria terminalis (Blomstedt et al., 2017). Finally, a study of 12 depressed patients showed a distinct pattern of oscillatory activity compared to that of obsessive-compulsive disorder patients when measuring local field potentials in the BNST during deep brain stimulation treatments (Neumann et al., 2014).

The evidence for the involvement of the stria terminalis in MDD and its potential as a therapeutic target for deep brain stimulation treatment suggests the need for a more precise neuroimaging technique to map and quantify the structure in-vivo. A reliable and valid anatomically driven diffusion-weighted protocol for visualising the stria terminalis could allow investigation of the white matter tract as a potential biomarker for depression as well as aid preoperative stereotactic localisation of the tract for deep brain stimulation treatments. No such protocol exists. To this end, I devised a deterministic diffusion protocol to extract the stria terminalis from the fornix (or fornix-stria combination). Essentially, this involved using the individual *FreeSurfer* 6.0 amygdala volumes generated from the amygdala study reported on in Chapter 4 to define what components of the fornix-stria combination were independent stria

terminalis fibres. To some extent, it can be considered a reversal of the technique described in Chapter 5 to isolate the fornix.

6.1.1. HYPOTHESES

My **hypotheses** were that

- 4) stria terminalis dimensional differences exist in depression
- 5) stria terminalis whole-tract diffusion metrics differences suggestive of tract pathology exist in depression, and
- 6) stria terminalis metrics differences suggestive of tract pathology exist in depression along only sections of the tract.

6.2. METHODS

The methods used for this study are described in detail in Chapter 2 (sections 2.2., 2.3., 2.4., 2.5., 2.6., 2.7., and 2.9). A brief summary description is given below.

Thirty people with Major Depressive Disorder (MDD) were compared to thirty healthy controls without MDD. All participants completed MINI (Mini International Neuropsychiatric Interview) for DSM IV (American Psychiatric Association, 1994) and Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960). All patients required a current MINI diagnosis of MDD with a HAM-D greater than 20. Controls were required to have no active or previous MINI diagnosis and a HAM-D less than 8. Only thirty from each of the original MDD and control groups were chosen for investigation using the stria terminals protocol due to time constraints (each stria took approximately an hour or more to clean (see below). Thirty MDD patients were chosen at random, and matched for age and sex with thirty controls.

6.2.1. MRI ACQUISITION

All data was acquired on a Philips (Best, Netherlands) Intera Achieva 3.0 Tesla MR system (32-channel head coil) at Trinity College Institute of Neuroscience, Dublin.

T1. 180 axial high-resolution T1-weighted anatomical images were acquired (T1W-IR1150 sequence, TE = 3.8 ms, TR = 8.4 ms, FOV 230 mm, $0.898 \times 0.898 \text{ mm}^2$, in-plane resolution, slice thickness 0.9 mm, flip angle $\alpha = 8^\circ$).

Diffusion Imaging. Whole brain, High Angular Resolution Diffusion Imaging (HARDI) (Jones and Leemans, 2011, Jones et al., 2013b) was acquired using a spin-echo echo-planar imaging (SE-EPI) pulse sequence (TE = 52 ms, TR = 11,260 ms, flip angle $\alpha = 90^\circ$), FOV 224 mm, 60 axial slices, 2mm^3 isotropic voxels, b-value = 1500 s mm^{-2} in 61 non-collinear gradient directions. This scan lasted 16 minutes.

6.2.2. IMAGE PROCESSING

STRUCTURAL

Cortical reconstruction and segmentation was performed using the *FreeSurfer* 6.0 image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>) (Fischl, 2012a, Fischl and Dale, 2000). The technical details of these procedures are described elsewhere (Fischl et al., 2002b, Desikan et al., 2006, Fischl et al., 1999, Fischl et al., 2004, Reuter et al., 2012, Dale et al., 1999). *FreeSurfer* interrogates T1 contrast differences between substructures using previously defined in-vivo and ex-vivo amygdala atlases to determine substructure characteristics. By selecting the 3T MRI flag and multispectral segmentation in *FreeSurfer* the procedure was optimized. The whole amygdala was reconstructed by summing all the amygdala output. Whole amygdala volumes were converted into masks for use in *ExploreDTI*.

DIFFUSION

Diffusion weighted imaging was corrected for signal drift, Gibbs ringing, eddy currents, head movement and processed according to the methods in Chapter 2. Tractography of the whole brain was generated using CSD modelling of all potential deterministic streamlines throughout the entire brain according to the following parameters; seed point resolution was set at 2 x 2 x 2 mm with a step size of 0.5mm, the FOD (Fibre Orientation Distribution) threshold was set to 0.1, and a maximum angle threshold set to 89 degrees. All steps were undertaken using the software *ExploreDTI*.

6.2.3. TRACTOGRAPHY

Both amygdala masks and whole brain tracts were randomized using a random number generator (<https://stattrek.com/statistics/random-number-generator.aspx>) so the individuals doing the manual tractography and fornix/stria extraction and cleanup were blind to the age, sex and group of each subject. This randomization was undertaken to prevent bias.

A Boolean logic protocol isolated a fornix-stria combination using AND and OR gates within *ExploreDTI* (see Chapter 2.7.) in each of the 60 (30 controls and 30 depressed) randomised subjects. As the amygdala is highly connected with multiple cortical and subcortical areas, this step was undertaken to limit the number of potential amygdala generated diffusion streamlines to those of the fornix and stria.

The stria terminalis is the primary output from the amygdala (Cassell et al., 1999). Using the 'Calculate tracts from folder of masks' option both bilateral amygdala volume masks (generated above from *FreeSurfer*) were used as combined AND gates with the fornix-stria tracts. This generated a group of streamlines from the fornix-stria that only included the stria terminalis, and excluded the fornix bilaterally (see Figure 6.3.). The stria terminalis was defined for the purposes of consistent analysis as from just above the anterior commissure to the mid-section of the crus (where the

crus is travelling completely superiorly). The 'tract splitter' tool (Settings→Analysis→Splitter Tool) was used to cut off fornix fibres peripheral to these points and preserve the fibres between. Note the main difference between calculating the stria terminalis and the fornix is the change of the amygdala from a NOT gate to AND gate (See Chapter 5.2.3).

The next step required that streamlines not associated with the fornix be removed. Cleanup was achieved with two raters (medical students Anurag Nasa and Caoimhe Gaughan) and a neuroanatomist from the Department of Anatomy at Trinity College Dublin after an extensive training period. Examining every subject individually, the raters collectively excluding streamlines not considered stria terminalis fibres using appropriately placed NOT gates. Criteria for exclusion included streamlines deviating from the known arch and loop shape of the stria terminalis and streamlines consistent with known adjacent tracts. To address potential bias, the neuroanatomist and raters were blind to the identity of each subject. These NOT gates were variable according to each subject and depended on the individual neuroanatomy of the region. However, commonly excluded streamlines included fibres from the hippocampal commissure, lyra, anterior commissure, the median forebrain bundle, stria medullaris and amygdalofugal pathway and various inter-thalamic connections. A second neuroanatomist reviewed a random subsample of 25 tracts from both raters to check face validity of the stria terminalis (looking at start and end points, the arch shape, the tract's relationship to other structures on MRI images (thalamus, anterior/posterior commissures and third ventricle) as well as the overall general impression of the tract. The second neuroanatomist also checked both raters' tracts simultaneously to investigate if they were occupying approximately the same space. This was considered a sufficient subsample for quality control of interrater reliability.

Following cleaning, each stria terminalis tract was saved. Tract statistics including macro dimensions (length and volume), standard micro diffusion measures such as fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial

diffusivity (RD) and Westin diffusion measures [spherical diffusion (CS), linear diffusion (CL) and planar diffusion (CP)] were calculated on each whole-tract using *ExploreDTI*'s built in tract statistics plugin (*Plugins* → *Convert* → *Info of tract *.mat file(s) to *.txt*). Along-tract measures were also calculated using *ExploreDTI*'s along-tract analysis function. This involved two steps; uniform resampling of the tract into 28 sections and then summarising the metrics. 28 sections were chosen as the average tract length was found to be 56 mm and the diffusion voxel size of 2 mm² ($56/2 = 28$). Sampling the tract into any more sections than this will not reveal further information. See Chapter 2.7.3. for tract metric equations.

6.2.4. STATISTICAL ANALYSIS

Demographic information was analysed using the statistical analysis program SPSS 24 (<https://www.ibm.com/analytics/us/en/technology/spss/>). Independent t-tests (Student, 1908) were performed to examine between-group differences in age, and HAM-D for controls versus MDD.

TRACT AVERAGED STATISTICS

A series of repeated-measures ANOVA (analysis of variance) controlled for age, sex and eTIV (estimated total intracranial volume) was used with left and right hemisphere diffusion microscopic metrics (FA, MD, AD and RD) and macroscopic tract measures (length, volume and number of streamlines) entered as the within-subject factors and group as the between-subject factor. Descriptives for group means, SEM (standard error of the mean) and 95% confidence intervals (CI) were obtained for each metric.

ALONG-TRACT STATISTICAL INVESTIGATIONS

All data was inspected for outliers and extreme values via the *Explore* tool within SPSS. Multivariate ANCOVA (analysis of covariance) for each diffusion metric (FA, MD, AD and RD) was used to investigate the variation of each metric over the length of the tract of interest. Between-group p-statistics for each metric were extracted for

each section. Multiple comparison correction (MCC) was performed using False Discovery Rate (FDR) correction (Benjamini, 2010). To guard against the known negative effect of outlier and extreme outlier values on multivariate statistical tests, all outliers were 'winsorized' (Hastings et al., 1947), which essentially limits the outlier points to the calculated upper and lower 95% CI boundaries. This also ensured additional statistical power, as missing data did not factor in the subsequent "cleaned" data. Age, sex and eTIV was controlled for throughout the analyses.

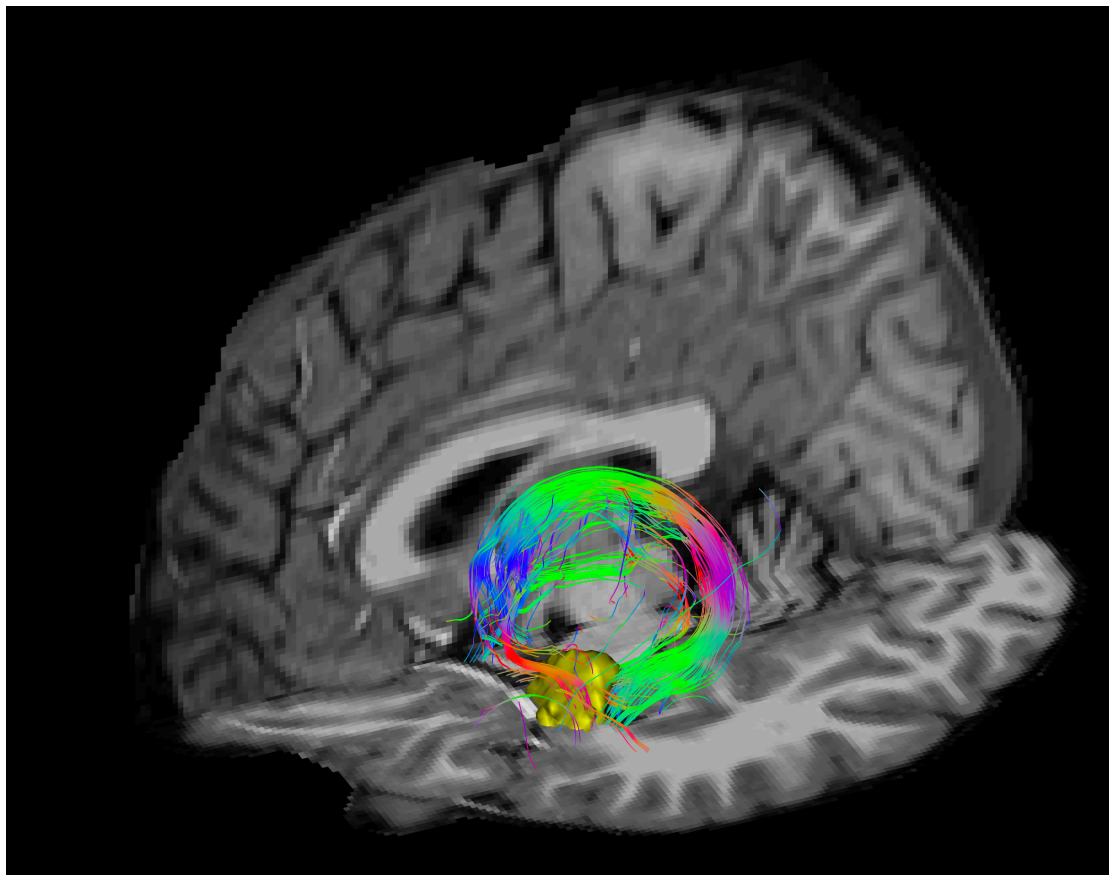


Figure 6.2. Extracted stria terminalis with amygdala in situ. Oblique sagittal and axial T1 image with the stria terminalis (pre-cleanup). Amygdala is light green in colour. Note the anterior commissure fibres in red travelling medially away from the amygdala.

6.3. RESULTS

DEMOGRAPHICS

Thirty patients and thirty controls were analysed for this study. Fifteen males and fifteen females with good quality diffusion imaging were chosen, within this limitation, at random in each group. Demographic information was analysed using SPSS 24 (<https://www.ibm.com/analytics/us/en/technology/spss/>). Independent t-tests (Student, 1908) were performed to examine between-group differences in age, and HAM-D for controls versus MDD.

No significant between-group differences between controls and depressed patients were found for age, sex or handedness. The average duration of depression in this subsample was 17 months. Both the age, HAM-D scores, handedness and duration of depression were representative of the full (80/83) sample (see Chapter 3, Table 3.3). All thirty patients were taking antidepressants at the time of scanning, compared with 87% of the full depressed group.

	Group		Con v Dep
	Con N=30	Dep N=30	p-value
Age: Mean(SEM)	29.0 (1.4)	31.2 (1.8)	0.13
Range (years)	16-64	17-64	-----
Male/Female (%M)	15/15, 50%	15/15, 50%	X ² : 1
Handedness (R/L)	13/2	14/1	NS
HAMD* (SEM)	1.3(0.2)	25.2(1.4)	5.10E-75
MDD months**	-----	17(6)	-----

Table 6.1 Demographic Data. Con, controls; Dep, depressed; HAM-D, Hamilton Depression scale; M, male; MDD, major depressive disorder; R/L, right or left handed; SEM, standard error of the mean.

6.3.1. TRACT VOLUMETRICS

Anatomical macroscopic tract comparisons revealed a significant main effect for tract length (F8.98 (1dof), $p=0.004$). Significant between-group differences were identified ($p=0.003$) in stria terminalis length with the MDD group having greater left ($p=0.012$) and right ($p=0.028$) fibre bundle lengths than controls (Figure 6.2).

									p-values		
		Left			Right			Within-subject/Interaction	ANCOVA		
	Group	Mean	SEM	95% CI	Mean	SEM	95% CI	Main effect F(dof), p: F(dof), p	ConvDep	Left	Right
Volume	Cons	1937.1	78.65	1670.15-1990.83	1995.8	72.56	1754.14-2231.86	0.1591(1), 0.69: 0.065(1), 0.80	0.161	0.229	0.329
	Dep	2075.71	78.65	1910.86-2302.14	2099.26	72.56	1991.36-2210.19				
Length	Cons	53.37	0.723	52.16-55.53	55.7	0.334	54.52-57.90	8.98(1), 0.004 : 2.373(1), 0.129	0.003	0.012	0.028
	Dep	56.1	0.723	54.55-55.79	56.8	0.334	55.99-57.75				
Lines	Cons	198.2	14.69	164.23-248.56	217.01	15.19	18737-270.63	0.693(1), 0.41: 0.448(1), 0.51	0.903	0.649	0.814
	Dep	207.94	14.69	188.74-262.63	211.8	15.19	185.676-250.67				

Table 6.2. Stria terminalis volumetrics. Volume, length and number of streamlines ANCOVA, analysis of covariance; CL, linear diffusion coefficient; Cons, controls; Cp, planar diffusion coefficient; Cs, spherical planar coefficient; Dep, depression; dof; degrees of freedom; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; SEM, standard error of the mean.

6.3.2. WHOLE-TRACT DIFFUSION METRICS

Independent repeated measures ANOVA, correcting for age, sex and eTIV, failed to identify any significant main effect for any diffusion metric or any significant interaction effect indicating that overall, the stria terminalis showed no difference in any diffusion metric between either left or right hemisphere. Between-group measures also failed to identify any group differences for any diffusion metric. However, the MDD group showed a trend towards FA reduction ($p=0.061$), with a strong trend of reduced FA ($p=0.057$) on the left (Figure 6.3).

6.3.3. ALONG-TRACT DIFFUSION METRICS

ANCOVA for along-tract metrics showed no significant differences on the right side between MDD and controls, after FDR correction. However, MD demonstrated an increase in four sections in the posterior section of the tract. RD showed an increase across eight continuous sections in the posterior third section of the left stria terminalis. See Table 6.4.

		Left			Right			Within-Subject/ Interaction Effect		ANCOVA	
DTI Metric	Group	Mean	SEM	95% CI	Mean	SEM	95% CI	Main Effect F(dof), p : Interaction F(dof), p	Between groups	Left (p, partial eta)	Right (p, partial eta)
FA	Cons	0.423	0.007	0.3927-0.4217	0.414	0.006	0.3965-0.4266	0.558(1), 0.46: 0.681(1), 0.41	0.061	0.057, 0.060	0.232, 0.24
	Dep	0.403	0.007	0.4060-0.4391	0.403	0.006	0.3943-0.4224				
MD	Cons	0.0012305	0.00002	0.0012-0.0011	0.0012083	0.000022	0.0011-0.0012	1.827(1), 0.182: 0.138(1), 0.71	0.742	0.89, 0.0005	0.66, 0.003
	Dep	0.0012343	0.00002	0.0012-0.0012	0.0012223	0.000022	0.0011-0.0012				
AD	Cons	0.0018402	0.0000234	0.0017-0.0019	0.0018051	0.000028	0.0017-0.0018	1.423(1), 0.238: 1.414(1), 0.24	0.996	0.57, 0.005	0.64, 0.004
	Dep	0.0018211	0.0000234	0.0017-0.0018	0.0018245	0.000028	0.0017-0.0018				
RD	Cons	0.0009241	0.00002	0.0008-0.0009	0.0009111	0.00002	0.0008-0.0009	0.159(1), 0.692: 0.952(1), 0.33	0.37	0.75, 0.002	0.24, 0.023
	Dep	0.0009324	0.00002	0.0009-0.0010	0.0009471	0.00002	0.0008-0.0009				
Westin											
CL	Cons	0.457	0.007	0.4291-0.4603	0.447	0.007	0.4391-0.4734	0.254(1), 0.616: 0.79(1), 0.38	0.16	0.101, 0.045	0.49, 0.008
	Dep	0.439	0.007	0.4285-0.4565	0.44	0.007	0.4255-0.4568				
Cp	Cons	0.081	0.002	0.0769-0.0863	0.081911	0.002248	0.0769-0.0863	0.42(1), 0.519: 1.987(1), 0.16	0.57	0.26, 0.021	0.94, 0.0005
	Dep	0.084	0.002	0.0788-0.0904	0.081652	0.002248	0.0788-0.0904				
Cs	Cons	0.461	0.006	0.4472-0.4768	0.47	0.006	0.4472-0.4768	0.867(1), 0.355: 0.482(1), 0.49	0.076	0.074, 0.053	0.24, 0.023
	Dep	0.478	0.006	0.4615-0.4867	0.48	0.006	0.4615-0.4867				

Table 6.3. Stria terminalis whole-tract diffusion metrics. FA shows a trend ($p=0.61$) for difference between groups, driven by a strong trend on the left ($p=0.057$). No other metrics show significance. AD: axial diffusivity; ANCOVA: analysis of covariance; CL, linear diffusion coefficient; Cons, controls; Cp, planar diffusion coefficient; Cs, spherical planar coefficient; Dep, depression; dof: degrees of freedom; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; SEM, standard error of the mean.

	Left					Right			
p-value (Con v Dep)									
Section	FA	MD	AD	RD	Section	FA	MD	AD	RD
1	0.479	0.709	0.835	1	1	0.541	0.8	0.567	0.727
2	0.048	0.163	0.532	0.06477	2	0.39	0.745	0.74	0.514
3	0.15	0.029	0.059	0.01323	3	0.813	0.761	0.935	0.602
4	0.135	0.012	0.065	0.00658	4	0.956	0.941	0.812	0.83
5	0.095	0.001	0.01	0.00354	5	0.516	0.863	0.42	0.768
6	0.263	0.01	0.03	0.00444	6	0.731	0.456	0.186	0.815
7	0.043	0.001	0.11	0.00581	7	0.529	0.172	0.084	0.073
8	0.04	0.005	0.344	0.00029	8	0.711	0.315	0.558	0.596
9	0.008	0.002	0.41	0.00002	9	0.933	0.36	0.302	0.212
10	0.01	0.063	0.56	0.00061	10	0.892	0.079	0.031	0.068
11	0.036	0.119	0.689	0.03433	11	0.913	0.411	0.564	0.659
12	0.01	0.415	0.885	0.13153	12	0.31	0.922	0.834	0.538
13	0.013	0.142	0.98	0.19949	13	0.237	0.748	0.806	0.614
14	0.008	0.523	0.842	0.94035	14	0.093	0.538	0.896	0.37
15	0.031	0.699	0.751	1	15	0.027	0.238	0.603	0.12
16	0.027	0.798	0.538	0.7686	16	0.002	0.073	0.252	0.035
17	0.023	0.781	0.238	0.50309	17	0.052	0.036	0.082	0.03
18	0.082	0.705	0.334	0.54953	18	0.291	0.035	0.046	0.045
19	0.368	0.524	0.297	0.48137	19	0.583	0.143	0.137	0.194
20	0.602	0.352	0.221	0.27609	20	0.303	0.088	0.154	0.136
21	0.784	0.408	0.295	0.25271	21	0.232	0.058	0.328	0.033
22	0.709	0.24	0.294	0.2335	22	0.407	0.044	0.277	0.04
23	0.458	0.178	0.163	0.25377	23	0.407	0.059	0.218	0.046
24	0.222	0.161	0.165	0.38607	24	0.735	0.012	0.018	0.068
25	0.472	0.14	0.234	0.16492	25	0.403	0.076	0.026	0.083
26	0.474	0.949	0.343	0.70679	26	0.205	0.042	0.115	0.029
27	0.042	0.176	0.353	0.20882	27	0.102	0.054	0.09	0.05
28	0.177	0.481	0.89	0.33989	28	0.607	0.057	0.111	0.081
Tract Average	0.063	0.658	0.79	0.41475	Tract Average	0.265	0.604	0.567	0.586
FDR	0	0.005	0	0.01323		0	0	0	0

Table 6.4. Stria terminalis along-tract diffusion metrics. Only RD and MD on the left survive strict FDR correction (highlighted in red). Bold indicates significance without FDR correction ($p < 0.05$). No metrics survive FDR on the right. Even with the less strict Bonferroni correction (Bonferroni = $0.05/28 = 0.002$), only one tract segment on the right survives. AD; axial diffusivity; CI, confidence interval; Con, controls; Dep, depression; FA, fractional anisotropy; FDR, false discovery rate; MD, mean diffusivity; RD, radial diffusivity.

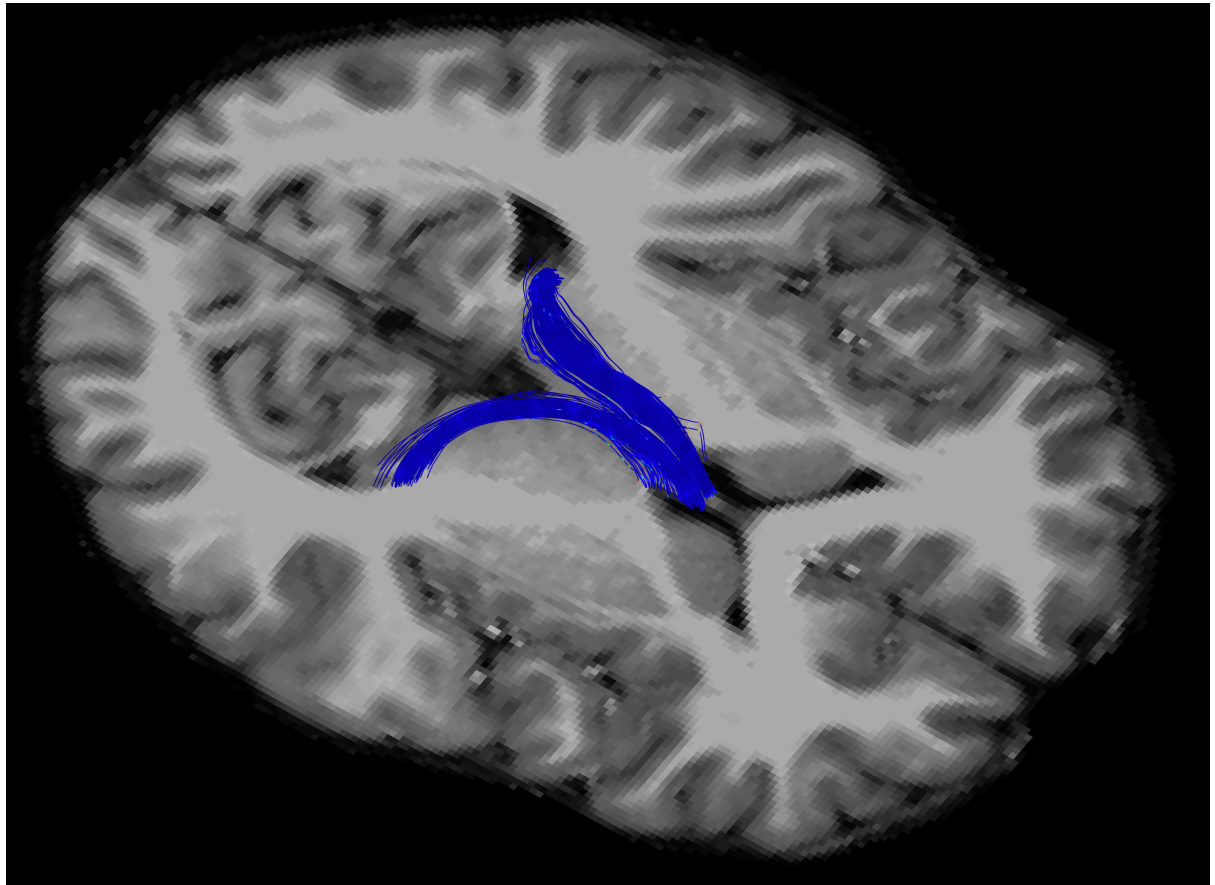


Figure 6.3. Bilateral reconstructed and cleaned stria terminali. Axial T1 image showing left and right stria terminali.

6.4. DISCUSSION

The stria terminalis showed no difference in volume or number of streamlines between depressed patients and controls. The stria terminalis was longer on both the left and right side in patients. The tract metrics AD, RD, and MD revealed no differences bilaterally in whole-tract analysis in MDD compared to controls. Depressed patients showed an overall trend towards FA reduction ($p=0.061$). This was driven by a strong trend towards reduced FA ($p=0.057$) in the left stria terminalis. Further investigating this difference using along-tract metrics found that this was driven not by FA in the tract, but rather driven by increased RD (average $p=0.0043$) and increased MD (average $p=0.002$) along the initial third section of the stria terminalis, corresponding to the left crus.

6.4.1. HYPOTHESIS 1: STRIA TERMINALIS DIMENSIONAL DIFFERENCES EXIST IN DEPRESSION

No differences were found in either volumes or number of streamlines between depressed patients and controls. The stria terminalis was longer ($p=0.003$) in MDD, both on the left ($p=0.012$) and the right ($p=0.28$). See Table 6.2.

As this is the first study to investigate the stria terminalis using this technique, I am unable to compare my results to other studies. In fact, to my knowledge, no other study or literature (including older pre-1900 books housed within TCD Department of Anatomy and TCD Early Printed Books and Special Collections) has reported the volumes and lengths of the stria terminalis using either cadaveric dissections or other imaging techniques. This longer tract demonstrated in MDD patients exists despite careful correction for estimated total intracranial volume, as defined using *FreeSurfer* T1 image analysis. This length increase most likely reflects differences in the distances between the anatomical landmarks used to segment this tract (the anterior commissure and mid-crus of the stria terminalis). The anatomical landmark variations in depressed patients may be due to shrinkage of the hippocampi bilaterally. Shrinkage of the hippocampi and other temporal lobe structures in depression could change (i.e. move more ventral) these landmarks used for segmentation of the stria terminalis. This needs further investigation and will include analysing temporal brain structures as well as basal forebrain areas ventral to the anterior commissure to examine reasons for a potential ventral-wise drop in anatomical landmarks. As part of my post-PhD work, I have already started to liaise with the Anatomy Departments in both TCD and UCD to measure stria terminalis (and other tract) dimensions. This will allow validation of these diffusion techniques, with particular focus on the appropriateness of the chosen anatomical landmarks. However, the similarity of volumes and generated streamlines between depressed patients and controls is reassuring as it shows that systematic bias when generating the stria terminalis was unlikely. The null hypothesis failed to be rejected in this case.

6.4.2. HYPOTHESIS 2: STRIA TERMINALIS WHOLE-TRACT DIFFUSION METRICS DIFFERENCES SUGGESTIVE OF TRACT PATHOLOGY EXIST IN DEPRESSION

Examining diffusion metrics for the entire length of the stria terminalis as a whole showed no differences in MD, RD and AD between depressed and controls, bilaterally. Overall, FA of the stria terminalis showed a trend towards being reduced ($p=0.061$). This was accounted for by differences on the left side ($p=0.057$). These did not survive FDR correction. See Table 6.3.

FA (fractional anisotropy) is a measure of diffusion along the principal eigenvector relative to the two other eigenvectors (See Chapter 2.7.3. for equations) and is considered a proxy measure of neuronal integrity (Alexander et al., 2007). Diffusion metrics are sensitive to several tissue characteristics (e.g. myelination, axon diameter, fibre density, fibre organization) and need to be interpreted carefully (Mori and Aggarwal, 2014). However, FA can be considered a *summary measure* of microstructural integrity and while highly sensitive to microstructural changes, it is less specific to the type of change. A reduced FA may be due to axonal degeneration or myelination issues, but in either case is suggestive of reduced connectivity along the tract. As the left stria terminalis connects the centromedial nuclei of the left amygdala to drive HPA-axis output, a reduced FA could reflect reduced connectivity between the left amygdala and hypothalamus.

The average FA was not reported in either of the two previously published studies investigating the stria terminalis in normal subjects using diffusion imaging (Kruger et al., 2015, Kamali et al., 2015b). Neither was it actually reported in the one study that investigated this tract in depression using diffusion imaging (Korgaonkar et al., 2014). That study however did report that depressed patients with a lower FA were more likely to achieve remission. No laterality was investigated. As my work is part of an eventual longitudinal follow up of these MDD patients, it may prove interesting to explore remission rates in our patients with respect to stria terminalis FA.

6.4.3. HYPOTHESIS 2: STRIA TERMINALIS METRICS DIFFERENCES SUGGESTIVE OF TRACT PATHOLOGY EXIST IN DEPRESSION ALONG ONLY SECTIONS OF THE TRACT

Along-tract analysis allows deeper exploration of diffusion differences. By dividing each stria terminalis into 28 equal sections (See 6.2. Methods, above), I was able to investigate the four principal diffusion metrics in each subsection. Section 1 (S1) was the start of the crus and section 29 (S29) was just above the anterior commissure (Table 6.4.). FA was reduced in MDD continually along the midsection of the left stria terminalis (S7-17), however, this failed to reach significance following FDR correction. Interestingly, both MD and RD were increased in continuous sections along the first third of the tract on the left side. MD showed a continuous increase from S2 to S10, reaching significance in four individual sections. RD showed a remarkable increase, sustaining significance following FDR correction across eight continuous sections (S3-S10). MD is the average of all three eigenvalues $[(\lambda_1 + \lambda_2 + \lambda_3)/3]$ and is an inverse measure of membrane density. It is also sensitive to cellularity, oedema and necrosis. However, generally speaking, similar to decreased FA, an increased MD may be a sign of axonal degeneration or myelination issues. RD is the average of the smaller two eigenvalues $[(\lambda_2 + \lambda_3)/2]$ and is much more sensitive to phenomena that 'widen' the diffusivity perpendicular to the main direction of the axons, i.e. it is inversely related to the density of axonal packing and the amount of myelination (Alexander et al., 2007). RD is also associated with general neuronal integrity, but less so than FA or MD (Tournier et al., 2011).

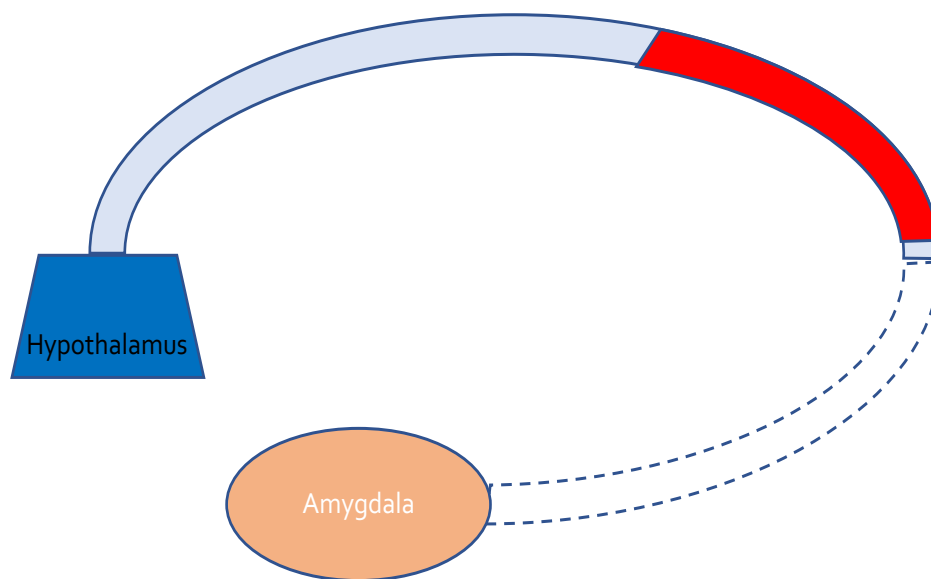


Figure 6.4. Schematic showing stria terminalis and affected tract portions. The dotted line is the part of the stria not generated in this study. The red section (posterior third) shows the portion of the tract showing increased RD. RD, radial diffusivity.

These results, especially the increased RD, are suggestive of problems with neuronal integrity within the first third of the left stria terminalis in depressed patients, possibly due to looser axonal packing, which commonly occurs due to demyelination. Looser axonal packing represents sparser versus more compact arrangements of axons within the tracts (Johansen-Berg and Behrens, 2013). Axonal packing density encompasses a variety of microstructural level variables (e.g., degree of myelination, axonal diameter, inflammation, etc.) that can influence the amount of extracellular water between axons. Increasing the extracellular water can increase the diffusivity of water perpendicular to the overall tract direction, increased RD. Depression has been associated with a pro-inflammatory profile (Doolin et al., 2018, Raison et al., 2006). Recent research implicates the pro-inflammatory interleukin-6 (IL-6 and anti-inflammatory interleukin-10 (IL-10) in the pathogenesis of depression. IL-6 is the most consistently elevated circulating cytokine in depression (Haapakoski et al., 2015, Pariante and Lightman, 2008), and reduces in response to antidepressants (Hannestad et al., 2011). A recent meta-analysis indicates that IL-10, an anti-inflammatory cytokine is elevated in depressed patients treated with

antidepressants, but not in anti-depressant free patients (Kohler et al., 2017). Potential inflammatory infiltrate in the first third of the left stria terminalis may account for the increased RD in MDD patients.

Demyelination in the first third of the left stria terminalis could account for the increased RD in MDD patients. Demyelination can be associated with MDD (Edgar and Sibille, 2012) and myelination has been proposed as mechanism for depression treatments (Bartzokis, 2012). Post-mortem studies have highlighted pathology of white matter in prefrontal brain regions related to decreased oligodendrocyte density and reductions in the expression of genes related to oligodendrocyte function (Tham et al., 2011, Uranova et al., 2004). Structural magnetic resonance imaging studies of MDD have also revealed deep white matter hyperintensities that are associated with clinical severity (Videbech et al., 2004), anhedonia (Dillon et al., 2018) and treatment responsiveness (Iosifescu et al., 2006, Papakostas et al., 2005). Demyelination is a common occurrence across many brain diseases (Love, 2006), and characterises multiple sclerosis (Reich et al., 2018). Depression is a well-documented complication in multiple sclerosis (MS) (Whitlock and Siskind, 1980), with up to 57% of patients suffering from comorbid MDD (Feinstein, 2007). Depressive symptoms in MS are considered most likely to be due to the organic white matter lesions (Zorzon et al., 2001, Schiffer and Babigian, 1984). Temporal lobe involvement in multiple sclerosis is a common feature when MDD is a comorbid condition (Zorzon et al., 2001) and could account for the depressive symptoms. Indeed depressive symptoms have been considered to be a prodromal sign for relapse of the neurological symptoms of MS (Shail, 2017).

While whole-tract analysis has value in providing general information about a tract, I have shown here (and in my fornix analysis (Chapter 5) that along-tract analysis can reveal deeper and more localised information on specific regions of the tract. The important differences found here using along-tract analysis (i.e. consistent variances along the proximal third of the tract on the left) would otherwise go unnoticed and

unreported if analysis was confined to whole-tracts only. Along-tract analysis is a relatively new technique (Colby et al., 2012) and only fifteen published studies have used this method in investigating any tract. No published study has used along-tract analysis of any tract in MDD, although one tract investigating mood disorders specifically post traumatic brain injury noted reduced FA in the anterior segments of the corpus callosum (Spitz et al., 2017). It is entirely possible that tracts previously identified as 'normal' in MDD may in fact hide localised changes similar to the changes demonstrates in this thesis

6.4.4. STRENGTHS AND LIMITATIONS

This is the first study to consistently extract the stria terminalis from whole brain tracts using deterministic tractography. Deterministic tractography is an experimental method that is subject to some technical challenges (Jones, 2008). There are known issues involving curving and kissing tracts (see Chapter 2.6.2.). The stria terminalis is highly curved and turns 180° in a relatively tight space. This was addressed by reconstructing the stria terminalis using high angle (89°), small step-size (0.5 mm) whole brain tracts. This tract lies deep within the centre of the brain, surrounded by and touching other white matter bundles; the fornix, stria medullaris, and various thalamic connections. CSD was chosen as the most appropriate tractography technique due to its superior ability at detecting complex crossing, contact and kissing fibres in a recent analysis of fibres estimation approaches (Wilkins et al., 2015). As with all diffusion-weighted reconstruction, the generated stria terminalis only describes the diffusivity in the region of interest and it is assumed that this corresponds to an actual tract (Mori and Aggarwal, 2014). A subsample of generated stria terminali (50%) was presented to a second neuroanatomist which showed good face validity of the results. To address this further I plan to systematically measure the length and curvature of a number of cadaveric stria terminali to determine baseline dimensions of this tract for comparison.

An experienced neuroanatomist with significant expertise of the region also aided during the cleanup. I felt this represented the most valid approach for removing extraneous streamlines. As the protocol was designed to incorporate as many of the potential stria terminalis streamlines as possible in a consistent manner, the initial gates were deliberately overly broad. This resulted in a large amount of cleanup, with the average time spent on cleanup (both rater and neuroanatomist working together) estimated at over 20 minutes per subject. It is appreciated that this approach may not be available or practical for some research groups. As this protocol was generated and executed as part of medical student summer research projects (supervised completely by me), enough person power and trained expertise were available to implement the full protocol, despite the enormous time commitments. However, with adequate neuroanatomical training, this future limitation for replication of the protocol may be overcome, and a researcher/technician experienced with *ExploreDTI* and the complex regional anatomy could single-handedly undertake the entire process.

Another limitation of this study (a similar limitation in the fornix protocol, see Chapter 5.4.4.) is that the posterior portion of the tract was clipped due from the crus downwards (see diagrammatic Figure 6.4. See also Figure 1.11. for diagram showing both fornix and stria terminalis tracts). This was done to ensure consistent anatomical landmarks (the anterior commissure and mid-crus) when segmenting out the stria terminalis, and also to have a similar protocol to the fornix for simplicity reasons. The beginning of the stria terminalis anatomically is the amygdala, which lies just anterior to the hippocampus. The significant portion of the tract between the amygdala and the crus which travels adjacent to the hippocampus until it reaches the crus is not measured in this thesis. The generated tract considered here is the dorsal arched-shaped section only. Considering the remarkable changes in RD found along the posterior third of the left stria terminalis, it would be interesting and worthwhile to generate the nearby sections of the tract directly all the way along to the amygdala. This will form part of my post-PhD work.

6.5. CONCLUSION

The stria terminalis forms the principal output tract from the centromedial amygdala to the hypothalamus and, if one considers the natures of the experiences in MDD, is likely to be involved in these experiences. In this chapter I demonstrated how I created a neuroimaging protocol to reconstruct the stria terminalis. This involved a novel technique using a previously generated amygdala from *FreeSurfer* to define the stria terminalis fibres. Using this technique, I was able to demonstrate differences in the stria terminalis between depressed patients and controls. The length of the stria terminalis was found to be reduced bilaterally in depression and diffusion metrics showed trend level decreases in the FA along the whole-tract on the left. Using along-tract analysis for further exploration of the different section, the proximal (to the amygdala) third of the tract showed increased MD and RD consistent with looser axonal packing that could possibly be related to demyelination or inflammation. Global decreases in FA and focal increases in MD and RD are suggestive of reduced connectivity of the amygdala to the hypothalamus in MDD. As the left amygdala is more associated with positive emotions than the right (see Chapter 4), reduced connectivity of the amygdala to the hypothalamus on the left compared to the right in MDD is consistent with right amygdala dominance in depression.

CHAPTER 7

CONCLUSIONS:

PUTTING IT ALL TOGETHER

"Every new beginning comes from some other beginnings end."

Seneca the younger
4BC – 65 AD

Major depressive disorder (MDD) is a debilitating mood disorder and is one of the leading causes of disability worldwide (Vos et al., 2012, Lépine and Briley, 2011). About 450,000 people in Ireland are affected by this common condition at any given time (Aware, 2017). Understanding the biological mechanisms underlying this disease may lead to clearer diagnostics and improved treatment for this disease.

In this thesis, I compared a total of eighty-three patients with MDD to eighty controls. As well as clinical interview, all participants underwent MRI (magnetic resonance imaging) scans to investigate the two key hubs of the limbic system: the hippocampus and the amygdala, involved in memory (Andersen, 2007) and emotion (Sah et al., 2003) respectively. These structures are fundamental to the coherent development, recognition, maintenance and expression of emotional systems and states. Due to the exceptionally extensive connectivity of these regions throughout the limbic system and with the rest of the brain, I also examined the main output tracts of the hippocampus and amygdala, the fornix and stria terminalis respectively. My methods are described in detail in Chapter 2.

The work has demonstrated the importance of these regions and their output tracts in MDD. The findings reported in Chapter 3, using novel anatomically driven reassemblies of *FreeSurfer* output, were that hippocampal volumes were smaller on the left in depression. The findings of Chapter 4 were that amygdala volumes were increased in key stress modulating areas on the right in the depressed patients compared to controls, and that amygdala asymmetry may have a role in the emotional experiences in MDD. In the same chapter, I also presented evidence that the cortisol awakening response (CAR), a measure of the stress response, was associated with amygdala volume differences in depression. In Chapters 5 and 6 I demonstrated site-specific involvement of the output tracts with both the right fornix and left stria showing differences in MDD compared to healthy controls.

In this final chapter, I will discuss (1) the **new techniques** used in the studies, (2) the findings in relation to the **hypotheses** outlined in Chapter 1 and Chapters 3-6, (3) a synthesis of the findings and how these findings add to the **international literature**, and finally (4) potential **future directions** for this research.

7.1 NEW TECHNIQUES

In this study I started used already established neuroimaging techniques and then further developed them to aid investigations of the limbic system in MDD. Methodological advances demonstrated in this thesis include innovative reassembly of *FreeSurfer* 6.0 substructures into anatomically relevant hippocampal and amygdala composites and a novel method of separating the fornix and stria terminalis tracts in diffusion imaging.

7.1.1. *FREESURFER* COMPOSITES

Using the computed substructures from *FreeSurfer* 6.0's hippocampal and amygdala modules, substructures were reassembled into useful composites based on the literature of the microstructure and the functional anatomy of the hippocampus and the amygdala. To my knowledge, this method is unique, in that there are no published studies that have used this reassembly technique to complement *FreeSurfer* output. Rather than just reporting the results of single substructures, the hippocampus volumes were examined using precise anatomical definitions, specifically the hippocampus proper, the hippocampal formation and the hippocampus extended (see Chapter 3.4.2. and Figure 3.3. and Figure 3.4.). Specifically, I have shown that hippocampal definition is important when interpreting changes in MDD. Also, by using exploratory composites, these novel reassemblies have uncovered interesting differences between the MDD and the healthy control groups (See Chapter 3.4.5. and Figure 3.6.). For example, by examining dentate and CA areas together yielded a most significant volume difference overall in MDD

($p=0.0001$), with these reductions driven by both first presentation and recurrent groups. The amygdala nuclei output were also used to generate three functional amygdala groups: the input regions, superficial regions and output regions. Similar to the hippocampal composites, these amygdala composites helped clarify the key role of the centromedial group in MDD (see Chapter 4, Table 4.7).

7.1.2. SEPARATING THE FORNIX AND STRIA TERMINALIS

The fornix and stria terminalis are closely aligned structures along most of their course. This poses substantial problems in separating these two structures using diffusion imaging (see Chapter 2.7. for a detail explanation). Previous deterministic and probabilistic diffusion methods have not succeeded in unravelling the fornix and stria from each other. To address this issue, I used the whole amygdala for each participant (previously generated) to preferentially extract stria terminalis fibres only from the fornix-stria combination, generating a section of pure fornix (i.e. hippocampal only) and a section of pure stria terminalis (i.e. amygdala only) tracts. To my knowledge this is the first time this technique has been used to distinguish between these two tracts. Using this novel approach, I have been able to demonstrate discrete differences between the fornix and stria terminalis in depression, i.e. the fornix shows differences on the right side anteriorly and the stria shows differences on the left side posteriorly. In a prior evaluation of the joint fornix-stria before applying the separation technique there were no apparent differences between depressed patients and healthy controls. This conflation of the fornix and stria terminalis could account for the lack of differences found in previous studies of the fornix in depression

7.2. AIMS AND HYPOTHESES

The two key limbic structures, the hippocampus and amygdala and their principal output tracts were compared in a group with MDD and a healthy control group. This was achieved by comparing cases to controls using state of the art hippocampal and amygdala segmentation and diffusion imaging techniques. Two broad aims and a total of fifteen structure-specific hypotheses were explored in this thesis.

7.2.1. AIMS

1. *differences exist between depressed patients and controls for each of the four structures when these structures are measured as whole/complete structures.*

Differences were found in MDD patients for the whole hippocampus (smaller bilaterally, but more so on the left), the whole amygdala (increased left-right asymmetry), the whole fornix (decreased fractional anisotropy (FA) on the right side), and the whole stria terminalis (longer bilaterally).

2. *differences exist between patients and controls for each of the four structures when these structures are examined at a more localised/substructural level.*

Differences were found in MDD patients for the hippocampal substructures (classic 'core' structures smaller in depression, with recurrent depression (RD) showing greater substructure change than first presentation depression (FPD)), amygdala substructures (centromedial nuclei), fornix tract sections (decreased axial diffusivity (AD) in the columns on the right) and stria terminalis sections (increased radial diffusivity (RD) and median diffusivity (MD) in the crus on the left).

7.2.2. STRUCTURE SPECIFIC HYPOTHESES

HIPPOCAMPUS (CHAPTER 3)

1. *Whole hippocampal volumes are smaller in MDD.*

Whole hippocampal volume differences exist in MDD, bilaterally, with the hippocampus smaller in MDD. **The null hypothesis was rejected.**

2. *The more specific the hippocampal definition (i.e. the core CA regions), the greater the degree of difference between MDD and controls.*

As the hippocampal definition became progressively more constricted from a general hippocampal structure to core cornus ammonis regions, the volume reductions seen in depression became more pronounced. **The null hypothesis was rejected.**

3. *Only the principal hippocampal substructures show differences in MDD.*

Substructure changes were found only in those structures considered part of the classic hippocampal formation (CA1-4, dentate and subiculum). **The null hypothesis was rejected.**

4. *Chronic MDD shows smaller hippocampi than first presentation MDD.*

Recurrent MDD showed both smaller whole hippocampal and a greater recruitment of affected substructures than first presentation depression. **The null hypothesis was rejected.**

5. *Exploratory composites can provide further information about MDD than just the individual computed substructures.*

Exploratory composites showed exceptional significance when combining the volume changes of CA and dentate regions, reinforcing the importance of these substructures in depression. **The null hypothesis was rejected.**

AMYGDALA (CHAPTER 4)

5. *Amygdala volume differences exist in MDD at a nuclear level but not at a whole amygdala level.*

The whole amygdala showed no differences in depression and the right medial nucleus was increased. **The null hypothesis was rejected.**

6. *Amygdala differences in MDD are predominately located in the centromedial nuclei.*

The right centromedial group volume was increased in MDD. **The null hypothesis was rejected.**

7. *Amygdala asymmetry occurs in MDD, reflecting the difference in emotional response (right – negative affect, left – positive affect).*

Depressed patients showed an increased left-right whole amygdala volume asymmetry, with larger differences between sides seen in MDD driven by increased right and decreased left amygdala volumes. **The null hypothesis was rejected.**

8. *The CAR, as a measure of chronic HPA axis overactivity, is negatively correlated with whole and nuclear amygdalar volumes in MDD.*

The CAR was negatively associated with both whole and amygdala nuclear volumes, bilaterally but more so on the right. **The null hypothesis was rejected.**

FORNIX (CHAPTER 5)

1. Fornix dimensional differences exist in depression.

The tract showed no difference in volume, length numbers of streamlines.

The null hypothesis was accepted.

1. *Fornix whole-tract diffusion metrics differences suggestive of tract pathology exist in depression.*

The right whole-tract showed decreased FA in depression. **The null hypothesis was rejected.**

2. *Fornix metrics differences suggestive of tract pathology exist in depression along only sections of the tract.*

The along-tract analysis showed significant decreases in axial diffusivity (AD) along the right column. FA was decreased in one section on the left column.

The null hypothesis was rejected.

STRIA TERMINALIS (CHAPTER 6)

3. *Stria terminalis dimensional differences exist in depression.*

The stria terminalis showed differences in length on both left and right sides, being longer in MDD. **The null hypothesis was rejected.**

4. *Stria terminalis whole-tract diffusion metrics differences suggestive of tract pathology exist in depression.*

The whole-tract showed no differences in any diffusion metric. **The null hypothesis was accepted.**

5. *Stria terminalis metrics differences suggestive of tract pathology exist in depression along only sections of the tract.*

The along-tract analysis showed significant increases in MD and RD along the left crus. **The null hypothesis was rejected.**

7.3. A SYNTHESIS OF THE FINDINGS

The key findings of the studies are shown in schematic form in Figure 7.2. In synthesising the findings, the significance of differences in each structure will be examined separately. This will be followed by a discussion of the symptoms and experiences of MDD in relation to the known neuroanatomical functions of the connections of the limbic system, and thus how my findings may be related to the phenomenological aspects of MDD. Finally, the laterality of the limbic system differences will be discussed.

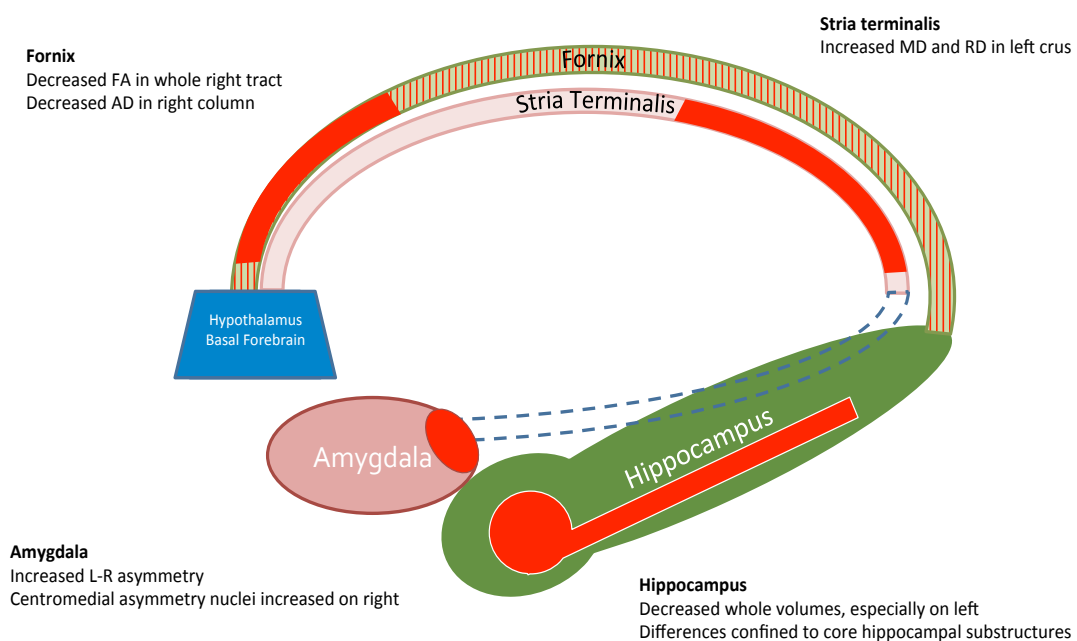


Figure 7.1. Schematic overview of main results. Affected areas are highlighted in bright red, or striped red. Highlighted areas are the core nuclei throughout the hippocampus, the centromedial amygdala nuclei, the anterior column of the fornix and the crus of the stria terminalis. AD, axial diffusivity; FA, fractional anisotropy; L-R, left-right; MD, medial diffusivity; RD, radial diffusivity.

7.3.1. STRUCTURES

THE HIPPOCAMPUS

Whole hippocampal volumes findings have been well described previously in MDD (Cole et al., 2011, Videbech and Ravnkilde, 2004, McKinnon et al., 2009). Even though changes in the deeper substructures have also been reported in MDD previously, the results have been inconsistent (Cao et al., 2017, Han et al., 2016). Multiple and occasionally unconnected substructures have been implicated in the pathogenesis of MDD (see Chapter 1, Table 1.). Using novel composite measures devised by exploiting the complexities of *FreeSurfer* output and assembled according to the known anatomical literature of the hippocampus, I have shown that hippocampal changes in MDD a) involve classic structures only (CA, dentate and subiculum regions), b) are particularly focused across the dentate/CA regions, c) are found only in CA2-4 in first presentation depression, and d) have extended to rest of the classic hippocampal structures in recurrent depression.

The involvement of the classic hippocampal processing subfields in depression may have profound implications for memory processing and cognition. As the primary processing subunit, the trisynaptic circuit is important for the normal flow of information through the hippocampus (Andersen, 2007). Volume reductions across the dentate and CA regions could impact the integrated functioning of all three components of the circuit (i.e. the perforant path, mossy fibres and Schaffer collaterals). In particular the CA2-4 regions were implicated in first presentation depression, in which the mossy fibre pathway runs (dentate/CA4 → CA3). These neurons have the unique property of neurogenesis (Abrous et al., 2005) and reduced neurogenesis secondary to stress has been suggested as a potential initial insult in MDD (Samuels and Hen, 2011). In particular the extension of pathology from CA2-4 in first presentation depression to encompassing all the classic core hippocampal regions in recurrent depression is highly suggestive of a progressive disease process in MDD. The volume reductions across the core processing circuitry could lead to difficulties in pattern separation and consolidation with global memory and cognition

impairments across multiple domains (Rolls, 2016, Grieves et al., 2017). These symptoms are consistent with those experienced by patients with MDD.

The hippocampus is also a key component of the HPA axis feedback loop. The hippocampus contains a high concentration of both glucocorticoid and mineralocorticoid receptors (Wang et al., 2013, Medina et al., 2013, Zhe et al., 2008), inhibiting corticotrophin releasing hormone release from the paraventricular nucleus of the hypothalamus, and shutting down the HPA axis. A potentially atrophied hippocampus could reduce this feedback inhibition, increasing HPA axis output. Hippocampal volume reduction has been proposed as a mechanism for HPA overactivation in MDD (Frodl and O'Keane, 2013).

In summary, these findings suggest a possible process whereby reduced hippocampal volumes, driven initially by CA2-4 reductions, and later by deficits across the classic 'core' processing centres in depression, may cause or contribute to HPA overactivation; as well as aberrant information processing resulting in the widespread memory and cognitive disturbances characteristic of MDD.

THE AMYGDALA

Whole amygdala volume differences have been much less consistently described in MDD (Hamilton et al., 2008). The amygdala has been considered a collection of diverse nuclei rather than as a discrete functional unit (Sah et al., 2003), and differences in the amygdala may be evident at a nuclear level rather than manifest in the amygdala as a whole. In this thesis I have found that although whole amygdala volumes were similar between groups that, a) nuclei differences existed but were localised to increases in the centromedial nuclei on the right, b) these right sided centromedial differences drove the increased whole amygdalar left-right asymmetry found in the MDD group, and c) amygdala volumes were associated with a flattened CAR.

The centromedial nuclei are the principal output nuclei of the amygdala (Swanson and Petrovich, 1998), directly interfacing with the stria terminalis and thus relaying amygdala output through this tract to the hypothalamus (Alheid, 2003). Stimulation of both the central and the medial nuclei have been shown in animal studies to intensify hypothalamically-driven autonomic responses and the HPA-axis (Jones et al., 2011, Myers et al., 2012). Right-sided increases in this nuclear group could suggest that the amygdala may be driving the increased HPA axis output found in MDD. The greater asymmetry found between the amygdalae in depression, with left-right whole amygdala volumes differences being greater in the MDD group, is consistent with the model of positive affect being mediated through the left amygdala (Lanteaume et al., 2007). The relative increase of the right amygdala to the left amygdala may reflect the dominance of negative feelings in patients with MDD. A flattened CAR is suggestive of a HPA axis in chronic overdrive, with chronic elevated cortisol release.

In summary, these findings together suggest that right amygdalar dominance, driven particularly by volume increases in right centromedial nuclei and associated with chronic HPA activation, may be the substrate for the overwhelming negative emotional experiences and intensified stress responses seen in MDD.

THE FORNIX

Fornix changes have been inconsistently described in MDD (Brisch et al., 2008, Frodl et al., 2012). This may be because differences in the fornix are isolated to particular sections only. In this thesis I have shown FA is reduced in the right fornix as a whole. Using along-tract analysis it was shown that this change is driven by decreased AD along the fornical columns on the right. AD is a proxy measure of neuronal health (Alexander et al., 2011).

The fornix is the main output tract from the hippocampus to the hypothalamus and basal forebrain regions (Carpenter, 1991, Andersen, 2007). Changes in neuronal

integrity or axonal damage in the fornix, as suggested by decreased FA and increased AD, may result in reduced connectivity of the hippocampus with the rest of the limbic system. This could have important consequences for global memory function. Through damage in the precommissural fibres of the fornix hippocampal integration with the motivational and pleasure centres in the basal forebrain may be impaired. This could interfere with the complex relationship between pleasant feelings and memory resulting in the reduced ability of depressed patients to recall or 'feel' past pleasant events. It could also result in the abridged encoding of pleasant events, leading to the reduced ability of depressed patients to associate normally pleasant events with pleasant feelings; again, symptoms of depression. Hippocampal inhibition of the HPA axis is also relayed through postcommissural fornix fibres. Neuronal damage in these fibres could reduce the hippocampal brake and result in overactivation of the HPA axis.

In summary, these findings suggest that irregularities in neuronal integrity along the fornix could impair memory, cause anhedonia, and increase the stress response in MDD.

THE STRIA TERMINALIS

Although the stria terminalis has been studied in depression, similar to the fornix, it has not been studied using the novel techniques employed in this thesis. I have shown that although no changes were seen in the whole stria terminalis tracts, along-tract analysis identified profound decreases in the RD and the MD measures in the left crus in MDD. RD and MD are proxy measures of axonal packing densities (Alexander et al., 2011).

As the major output tract of the amygdala (Mai and Paxinos, 2011), and directly interfacing with the centromedial amygdala, the stria terminalis transmits signals from the amygdala to the basal forebrain and to the hypothalamus. Activation of the left amygdala is associated with positive emotions. Reduced left amygdala output to

the motivational and pleasure centres in the basal forebrain may result in less positive emotional experiences. The corollary is that there may be relatively increased connectivity from the right amygdala to the HPA axis resulting in increased stress responses. Imbalance in the left-right amygdalar input to both the basal forebrain and the HPA axis occurs, with relative dominance of the more negatively emotional right side.

In summary, these findings suggest that axonal or myelination abnormalities along the left stria terminalis may result in poorer signals from the left amygdala leading to reduced communication of positive emotions to the pleasure areas and hypothalamus.

7.3.2. EXPERIENCES AND SYMPTOMS

MDD is characterised by the core experiences/symptoms of low mood (sadness) and anhedonia (lack of pleasure or interest), along with a combination of the other symptoms listed below that must persist for at least two weeks. Depression varies, and patients will often describe their own distinctive experiences without an ability to identify the experience. The DSM-5 experiences/symptoms of MDD are as follows (American Psychiatric Association, 2013) (see Chapter 1.1 for further details);

10. Depressed mood or irritable most of the day, nearly every day
11. Decreased interest or pleasure in most activities, most of each day
12. Significant weight change (5%) or change in appetite
13. Change in sleep: Insomnia or hypersomnia
14. Change in activity: Psychomotor agitation or retardation
15. Fatigue or loss of energy
16. Guilt/worthlessness: Feelings of worthlessness or inappropriate guilt
17. Concentration: diminished ability to think or concentrate
18. Suicidality: Thoughts of death or suicide, or has suicide plan.

The neuroimaging findings in the hippocampus, amygdala, fornix and stria terminalis in MDD found in my studies can be mapped onto the experiences of depression. The following constructs provide a coherent neurobiological explanation of depression symptomatology in relation to the limbic abnormalities found in my studies.

LOW MOOD

Disturbance of mood is the cardinal underlying symptom of major depressive disorder (American Psychiatric Association, 2013). An individual with depressed low mood characteristically has low mood most of the day and almost every day. Prevailing mood can be considered the sum of positive affect and negative affect (Davidson, 1998) and in major depressive disorder, negative affect is increased, while positive affect is decreased (Kring and Bachorowski, 1999). In depression, low mood is described as pervasive because objectively positive emotional events have little impact on the overall mood. In this study, I found that the normal amygdalar left-right asymmetry was more skewed towards larger right versus smaller left amygdalar volume in MDD. As the right amygdala predominantly mediates negative emotions (Liu et al., 2015), and the left amygdala is known to be involved in positive emotions (Lanteaume et al., 2006), the increased amygdala asymmetry could account for the pervasive low mood that is the core experience in MDD. Similarly, attenuated left amygdala output via a compromised left stria terminalis may further exacerbate right amygdala negative affective dominance. Persistent negative emotional “labelling” of sensory information from a dominant right amygdala could also account for the symptoms of low self-esteem, inappropriate guilt, as well as hopelessness.

ANHEDONIA & FATIGUE

Anhedonia, or lack of pleasure in interest, is the second core symptom of depression (American Psychiatric Association, 2013). Motivation and pleasure are mediated via multiple interrelated reward and disappointment circuits (Fakhoury et al., 2016, Dalley and Everitt, 2009, Roddy et al., 2018b), particularly involving dopamine

(Bromberg-Martin et al., 2010). The basal forebrain is fundamental across all these circuits. The basal forebrain contains the septal nuclei (Gottesfeld and Jacobowitz, 1979) and nucleus accumbens (Powell and Leman, 1976) reward centres. Both the hippocampus and the amygdala are connected to these areas through the fornix and stria terminalis respectively. Reduced left stria terminalis connectivity to the basal forebrain could limit the positive affective output from the left amygdala. Similarly, a damaged fornix may limit hippocampal integration with these regions, interrupting the intricate relationship between pleasant feelings and memory. Disconnecting the left amygdala and hippocampus from the basal forebrain could result in reduced reward and motivation, and an inability recall or 'feel' past pleasant events, furthering anhedonic experiences.

WEIGHT & APPETITE CHANGES

Weight loss and reduced appetite are common features of depression (American Psychiatric Association, 2013). Weight loss may be a consequence of hypothalamic output from the hunger and satiety centres or could result from increased CRH output (Arone et al., 1995). The relevant hypothalamic nuclei may be modified as a consequence of combined increased amygdalar and decreased hippocampal influences on the hypothalamus.

SLEEP DISTURBANCE & PSYCHOMOTOR AGITATION

Sleep disturbance is also common in MDD (American Psychiatric Association, 2013). The relationship between the hippocampus and sleep is well documented (Ferrara et al., 2012, Kreutzmann et al., 2015, Emrick et al., 2016). Sleep disturbance considered a potential initial mechanism in MDD (Germain and Kupfer, 2008, Kronfeld-Schor and Einat, 2012). However, sleep disturbance may be a consequence of HPA axis overactivation (Buckley and Schatzberg, 2005). Again, this could be caused by the amygdala and hippocampal changes, and/or the pathways from the hippocampus and/or the amygdala to the hypothalamus. Psychomotor agitation, another common depression symptom may be mediated through similar mechanisms.

LACK OF CONCENTRATION, POOR MEMORY & COGNITIVE SLOWING

Patients with depression often complain about concentration and memory impairments, and difficulties with working memory (American Psychiatric Association, 2013). Patients with MDD often describe feeling overwhelmed by previously simple tasks. Reduced cognitive flexibility and memory capacity may be caused by left core hippocampal circuitry impairment.

7.3.3. TAKING SIDES: A QUESTION OF BALANCE?

The laterality of the findings in MDD is interesting. In terms of processing centres; the hippocampus shows greater differences on the left while the amygdala shows greater differences on the right. Conversely, in terms of white matter tracts the fornix shows differences on the right while the stria terminalis shows differences on the left.

The right amygdala mediates negative emotions more than the left. The increase in right to left amygdala size in MDD in this study may account for the dominance of negative over positive affect experienced by patients with depression. This is mediated here through increased right centromedial nuclei volumes. Left stria terminalis compromise may further exacerbate this imbalance of negative versus positive affective emotional output to the hypothalamus by restricting left positive emotional output.

The left hippocampus is particularly involved in verbal and declarative memory (Maguire and Frith, 2003, Miller et al., 2018). The deficits identified here in the core processing units in MDD may result in the widespread cognitive and memory deficiencies experienced by individuals with depression. HPA overactivation is suppressed through hippocampal mechanisms (Dedovic et al., 2010, Frodl et al., 2014) and the reduced hippocampal size shown in this study may compound the already excessive hypothalamic mobilisation from the amygdala. Right fornix

compromise may further exacerbate the lack of HPA inhibition by restricting right hippocampal regulation of hypothalamus.

7.4. FUTURE DIRECTIONS

7.4.1 FUTURE DIRECTIONS USING THIS DATA SET

ANTERIOR-POSTERIOR AXIS SEGMENTATION

Since finishing my analysis of the hippocampal substructures, a new *FreeSurfer* 6.0 update has been released containing a new beta update of the hippocampal analysis module. One of the key features that this update has allowed us to do is to segment the hippocampus in the anteroposterior axis, effectively generating two further sets of substructures: an anterior group and a posterior group. Hippocampal research is becoming increasingly focused on the important anatomical and functional variability along the long axis of the hippocampus (Poppenk et al., 2013). This research suggests that differences in connectivity (Fanselow and Dong, 2010), organisation of grid cells (Stensola et al., 2012) and in particular substructure compositions (Malykhin et al., 2010, Duvernoy, 2005) between the anterior and posterior hippocampus can influence the role of the hippocampus in depression. The anterior hippocampus has abundant connections with adjacent amygdala (Smith et al., 2009) as well as connectivity to the ventromedial prefrontal cortex and insula via the uncinate fasciculus (Kier et al., 2004). These connections suggest a role for the anterior hippocampus as a focus point that may mediate the inputs of emotional activation from the amygdala against emotional inhibition from the ventromedial prefrontal cortex. This front part of the hippocampus is also functionally linked to the ventral striatum and midbrain ventral tegmental area (Krebs et al., 2011, Haber and Knutson, 2010), both brain regions involved in reward. The anterior subiculum also has strong connections with the HPA axis (O'Mara, 2005). Consequently, the anterior hippocampus has greater connectivity to the emotional networks than the posterior

part, which is instead more involved in cognition and memory via its connections with the thalamus, and cingulate regions (Poppenk and Moscovitch, 2011). CA regions take up a greater proportion of the anterior hippocampal regions (50%) compared to the posterior regions (38%) (Malykhin et al., 2010), suggesting that the core hippocampal differences that I found in MDD, compared to healthy controls, may be intensified anteriorly. Reanalysis of the data may show relatively greater differences between MDD and healthy controls in the anterior sections. This is underway.

STRUCTURE AND OUTPUT

In this thesis hippocampal and amygdalar structures and their principal output tracts were analysed separately. Further information could be gained by examining each structure with their output tract together, e.g., do hippocampal and fornix differences occur together and do amygdala and stria terminalis differences occur together in MDD? This was not done for time reasons, but such an analysis is planned.

Both the amygdala and hippocampus have three output tracts, but the stria terminalis and the fornix are the dominant output tracts respectively. The remaining hippocampal output tracts are the perforant pathway to the entorhinal cortex and the cingulum bundle connecting frontal and cingulate gyrus regions to the hippocampal and parahippocampal regions (Andersen, 2007, Andersen, 1975). Both the perforant path (Augustinack et al., 2010) and the cingulum (Jones et al., 2013a) have been studied with diffusion imaging. I have already started segmenting the cingulum into its four discrete components with a group of medical students. This process is due to be completed shortly. The remaining amygdala output tracts are the anterior commissure and the amygdalofugal pathway (Aggleton, 1992). It is also possible to reconstruct both of these tracts with diffusion imaging (Kamali et al., 2015b, Kamali et al., 2016). Considering that the amygdalofugal pathway also innervates the hypothalamus, similar to the stria terminalis, examining this tract with the cortisol measures may reveal further differences in depression.

A further project that is planned is the analysis of the specific substructures and nuclei together with the output tract from that nucleus or substructure. For the hippocampus this would be the CA1 and the subiculum, with the fornix (Andersen, 1975); and for the amygdala, it would be the centromedial nuclei and stria terminalis (Weller and Smith, 1982). Generation of these tracts would follow a similar method to that described in this thesis, using *FreeSurfer* 6.0 generated composite masks of the CA1/subiculum and centromedial structures as opposed to whole amygdala volumes used in this thesis. Such a refinement of the method may reveal even more specific components of the stria and fornix.

PRE AND POST COMMISSURAL COMPONENTS

Both the fornix and the stria terminalis demonstrated distinct pre and post commissural sections, as each tract split across the anterior commissure (See Chapter 5.4.4 and Figure 5.5.). Due to the clear divisions of the tract as they cross the commissure, it would be relatively easy to isolate either pre or post commissural fibres using targeted AND or NOT gates as appropriate. The precommissural fibres innervate the basal forebrain/septal areas and the postcommissural fibres innervate the hypothalamic regions. These areas have distinctive functions of pleasure/motivation and stress axis/memory/vegetative functions respectively. Investigating these tracts sections separately could uncover unique circuit specific changes in depression.

ADDITIONAL COMPOSITES

Based on the success of these methods, I moved on to look at additional composites along more functional lines. By adding in the entorhinal cortex output from *FreeSurfer* 6.0 (not studied in this thesis), it would be possible to create three new composites corresponding to each component of the classic trisynaptic circuit. The trisynaptic circuit allows entorhinal (EC) inputs to be processed through the key hippocampal subfields (Andersen, 2007; Stepan *et al.*, 2015). As the circuit's name suggests it consists of three synaptic connections, entorhinal cortex to dentate through the perforant path (EC → Dentate/CA4), dentate to CA3 through the mossy fibre

pathway, (Dentate/CA4 → CA3) and finally CA3 to CA1 through the Schaffer collaterals (CA3 → CA1) before finally outputting through the subiculum (See Chapter 1.3.2., Chapter 1.3.2., Chapter 1.3.3. and Figure 1.3, and Figure 1.5). This circuit is the fundamental processing unit of the hippocampus and impairment of any component could have global consequences for memory and cognition, symptoms of depression. These potential composites are visualised in Figure 7.1. and if examined could provide greater insight into the detailed circuitry changes that may occur MDD.

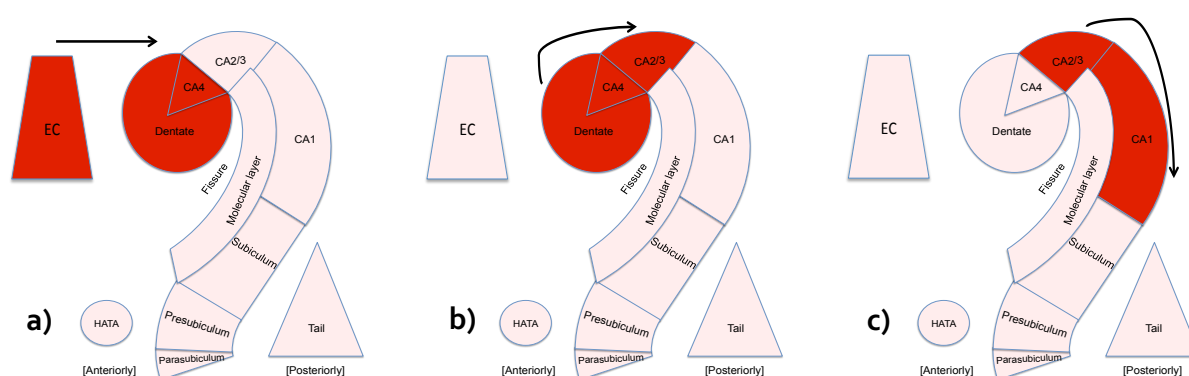


Figure 7.2. Proposed INPUT, MIDDLE, OUTPUT composites. The three proposed trisynaptic composites based on *FreeSurfer* 6.0 output. a) Entorhinal cortex to dentate through the perforant path (EC → Dentate/CA4); b) dentate to CA3 through the mossy fibre pathway, (Dentate/CA4 → CA3) and; c) CA3 to CA1 through the Schaffer collaterals (CA3 → CA1) before finally outputting through the subiculum. CA, cornu ammonis, EC, entorhinal cortex, HATA, hippocampal amygdala transition areas.

The exploratory hippocampal composites I have used (Complete Dentate, CA-only, Combined Dentate/CA and CA2-4; see Chapter 2.5.1. and Chapter 3.4.5. for further details) and the further trisynaptic composites that I have proposed may be used in other datasets to see if these focused anatomical hippocampal changes can be replicated. *FreeSurfer* 6.0 has been used to investigate bipolar affective disorder (Mitchell et al., 2018), schizophrenia (Li et al., 2018), psychosis (McHugo et al., 2018), obsessive-compulsive disorder (Reess et al., 2018), post-traumatic stress disorder (Chen et al., 2018), anorexia nervosa (Myrvang et al., 2018) normal personality (Gray et al., 2018), Alzheimer's disease (Sarica et al., 2018), Parkinson's disease (Radziunas

et al., 2018), primary biliary cholangitis (Mosher et al., 2018) and vitamin D deficiency (Al-Amin et al., 2018). *FreeSurfer* 6.0 has also recently been used to explore hippocampal volumes in MDD (Cao et al., 2017) and treatment response to electroconvulsive therapy (Gryglewski et al., 2018, Cao et al., 2018) (See Chapter 1.3.6. for further details). However, none of the above studies reassembled the hippocampal subfields into anatomico-functional groups similar to those presented in Chapter 3. Due to the deeper understanding of the specific hippocampal changes uncovered in patients with depression from using these composites, future use of similar substructural assemblies could aid in the greater understanding of the anatomico-functional hippocampal changes that may occur in other neuropsychiatric conditions.

Analysing the pooled amygdala and hippocampal data in one analysis, rather than analysing just individually may expose the relative changes that occur between these two structures in depression. As the hippocampus is smaller in depression, bilaterally, but more marked on the left, and the amygdala shows an overall greater asymmetry in MDD, greater on the right, it would be interesting to also look at right-left hippocampal asymmetry in patients with depression. Hippocampal right-left asymmetry was also not examined in this thesis and is planned to be analysed in the future.

Finally, over the past year - i.e. since collection of MDD data ceased - I have continued scanning controls of specified ages and sex to augment our control dataset to include a balanced representative sample of normal healthy individuals (15-65) over the lifespan. I plan to use *FreeSurfer* and my composite techniques to examine changes in the hippocampal subfields and amygdalar nuclei across different age groups. This normative data may provide insights into the normal development and aging of these limbic regions at a substructural level.

NEUROANATOMICAL CORRELATIONS OF DIFFUSION DATA

Diffusion MRI images the diffusion of water only; and tracts are then inferred from the diffusion properties based on prior neuroanatomical information (Tournier et al., 2011, Farquharson et al., 2013). Anatomical validation of the techniques employed in the studies would provide additional scientific weight to the measures and give more validity to the interpretation of the results. The first step towards a full anatomical validation would be to quantify the fornix and stria terminalis lengths and volumes from cadavers. Permission to do this has already been granted from Trinity College Dublin and University College Dublin and I am involved in a collaborative project with both institutions. We are in the processes of dissecting out the tracts and measuring the dimensional features of in cadavers. Matching the volumes and lengths from dissected specimens to those from in-vivo MRI will go some way to providing a reasonable face validity of the structure (accounting for brain shrinkage and other cadaveric factors). I used a similar approach to validate tractography of the stria *medullaris* with success and have published the results (Roddy et al., 2018c). The technical difficulties of diffusion imaging in cadaveric brains are outside the scope of this thesis (Tofts et al., 2008, Roebroeck et al., Miller et al., 2012), however two attempts at this have been made by myself to date, with limited success. I plan to liaise with a group at the Institute of Neurology, Queens Square, London who have had some success with this approach.

FPD AND RD AND PREDICTIONS FOR ALL STRUCTURES

For this thesis I divided the MDD cohort into first presentation depression (FPD) and recurrent depression (RD) groups for the hippocampus only. This was done only in Chapter 3 (out of the total four studies) due to time constraints. It would be worthwhile to investigate the amygdala in both FPD and RD patients, since the amygdala dataset is the same as the hippocampus. This is underway. Unfortunately, cortisol correlations in FPD and RD with amygdala volumes will not be possible due to power considerations (only 25 control subjects) but merits further investigation as a future project.

Similarly, I only examined the predictive effects of hippocampal and amygdala volumes and substructures on disease diagnosis and chronicity of depression. This identified two potential markers in MDD, with diagnosis predicted by the central and medial nuclei of the amygdala, and disease duration predicted by both the hippocampal CA1 subfield and amygdala centromedial asymmetry. Future analysis of the fornix and stria terminalis will involve similar hierarchical regression models to also investigate the predictive effects of these structures in MDD. This may reveal these white matter structures as potential biomarkers in depression.

CORTISOL

Liquid chromatography-mass spectrometry (LC-MS) was the method used for the analysis of cortisol concentrations in saliva. Our group is one of only a few that have used LC-MS for the measurement of cortisol. LC-MS has many advantages over the traditional method of ELISA (enzyme linked immune-sorbent assay) . It allows the measurement of cortisone as well as cortisol from saliva (Antonelli et al., 2015, Owen et al., 2010), and there is negligible inter-assay variability. Cortisone is converted to cortisol via the enzyme 11 β -HSD1 and has about 80% of the activity at glucocorticoid receptors as cortisol (Raff and Findling, 2010). Polymorphisms of 11 β -HSD1 have been implicated in human HPA axis regulation and in subject susceptibility to depression (Dekker et al., 2012). Reanalysis of the data taking into account the cortisol/cortisone ratio could reveal further interactions of the neuroimaging findings with the HPA axis findings. Future studies should involve other hypothalamic output measures, including autonomic nervous system responses, for example, the measurement of sympathetic skin responses (Zygmunt and Stanczyk, 2010), heart rate variability (Bassett, 2016) or sleep cycle alterations (DeMasi et al., 2017). These physiological parameters are known to be affected in depressed patients and could be measured with relative ease using modern wearable mobile technology (Sano et al., 2015).

SYMPTOMS

Major Depressive Disorder is a cluster of symptoms rather than a uniform clinical entity (see Chapter 1.1). All the patients in this study had a DSM-IV diagnosis of MDD and a HAM-D score of greater than 17. Each patient had an individual symptom profile that was captured in the HAM-D and the Structured Clinical Interview. More specific patient experiences were also captured with a sleep scale, the Pittsburgh Sleep Quality Index (Buysse et al., 1989), the Hamilton Anxiety Scale (Hamilton, 1959) to probe anxiety symptoms and ruminative thoughts using the Rumination-Reflection-Questionnaire (Trapnell and Campbell, 1999) (See Appendix 3 for scales). Importantly, childhood adversity was also measured using the validated Childhood Trauma Questionnaire (Bernstein et al., 2003). These measures have not been incorporated into the analysis in this thesis. However, going forward, with these documented symptoms, it would be possible to correlate specific symptoms with neuroimaging and HPA axis measures. This approach may help uncover some of the specific mechanisms of the particular experiential components of patients with depression.

7.4.2 FUTURE DIRECTIONS USING THE METHODS DEVELOPED IN THE THESIS

LONGITUDINAL ANALYSIS

This thesis studied the neuroanatomical characteristics of MDD in FPD and RD groups. As discussed as a limitation in Chapter 3.4.6, these are distinct groups of individuals, and this is not a longitudinal analysis. As correlation does not equal causation, it is impossible to infer disease progression between the two groups based on this data. The patients in both groups have been invited back for further testing and it is hoped that enough patients return to allow sufficient power to investigate the effects of disease progression or recovery between the two timepoints. As of this thesis, 10 patients with FPD have been rescanned (average time since the primary scan is about 2 years). Most of these patients (8 out of 10) have recovered completely from MDD and unfortunately, it is not possible to infer disease progression from

these. I am currently in the process of attempting to re-engage with FPD group to facilitate rescanning.

HIPPOCAMPAL AND AMYGDALAR COMPOSITES AND OTHER DATA

By defining the whole hippocampus from 'the ground up' through assembling substructures into hippocampal proper, hippocampal formation or hippocampal extended (Chapter 2 and Chapter 3) this thesis has demonstrated the importance of whole hippocampal definitions. Hippocampal research has been mired in an overabundance of competing and occasionally conflicting definitions of whole hippocampal volumes (Geuze et al., 2005) with up to sixty differing definitions over the years in previous research. Variable hippocampal volumes across research groups give rise to significant complications in inter-study interpretations. A composite-based approach, as I have proposed may resolve these inconsistencies moving forward. Examples of conditions which have shown hippocampal involvement include bipolar disorder (Cao et al., 2017), post-traumatic-stress-disorder (PTSD) (Averill et al., 2017), Alzheimer's disease (Mueller et al., 2007). Precisely defining the hippocampus (as either hippocampal proper, hippocampal formation or hippocampal extended) and using other exploratory composites to investigate anatomico-functional components of the hippocampus may shed further light on the pathophysiology of these diseases. Similarly, the three anatomical composites of the amygdala (laterobasal, centromedial and superficial) may allow a richer investigation of the role of the amygdala in diseases such as PTSD (Morey et al., 2012), frontotemporal dementia (Boccardi et al., 2002), schizophrenia (Garcia-Marti et al., 2008) and anxiety disorders (Wiest et al., 2006).

HIPPOCAMPAL AND AMYGDALAR OUTPUT AND OTHER DATA

The novel approach to separate the fornix and stria terminalis as shown in this thesis, may be applied to other conditions though to involve hippocampal and amygdalar function and in particular output. Accurate separation of these two previously 'conjoined' structures in neuroimaging may further greater precision of the role of these structures in disease. Such fornical associated conditions include Alzheimer's

disease, (Tang et al., 2017), normal pressure hydrocephalus (Hattori et al., 2012) multiple sclerosis patients (Dineen et al., 2009) and schizophrenia (Fitzsimmons et al., 2009). Stria terminalis associated conditions include generalised anxiety disorder (Buff et al., 2017) and PTSD (Miles and Maren, 2019).

7.5. CONCLUSION

The findings in this thesis indicate the importance of the key limbic regions of the hippocampus and amygdala and their main output tracts in major depressive disorder. Using advanced neuroimaging and novel analysis techniques I have shown that (a) the general finding of smaller hippocampal volume in MDD is likely to be due to changes in the core processing areas of memory formation, rather than the extended hippocampal regions; (b) the difference in the affective roles attributed to the left and the right amygdala may be reflected in actual asymmetry of the amygdalae in MDD, and that this may be driven by the centromedial stress-modulating nuclei; (c) chronic HPA overactivity is associated with larger amygdala volumes in depression, (d) both the fornix and stria terminalis were different in MDD compared to healthy controls at specific sections of the tracts; and (e) the hippocampal CA1 subfield and amygdala central/medial nuclei may have predictive ability in depression. These findings may account for the symptoms found in MDD such as anhedonia, low mood, poor concentration and vegetative disturbance. Further research refining, verifying and developing these new techniques will be necessary to confirm these findings. In the future, I will be applying these techniques and others across the entire REDEEM dataset to further investigate the neurobiology of MDD. I also plan to investigate this dataset longitudinally to investigate the effects of disease progression or recovery on these limbic structures.

CHAPTER 8

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"If I have seen further than others,
it is by standing on the shoulders of giants."

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APPENDIX 1.1. POSTERS

1. The human dorsal hippocampal commissure: using high-resolution tractography to delineate the afferents traversing this midline structure

Gaughan C, Nasa A, O'Keane V, **Roddy DW**

Neuroscience Ireland, Dublin 2018

2. Atlas-based and manual diffusion tractography of the fornix – a comparison

Drago T, Martin A, Gaughan C, Nasa A, O'Keane V, **Roddy DW**

Neuroscience Ireland, Dublin 2018

3. Papez's forgotten tract: 80 years of unreconciled findings concerning the thalamocingulate tract

Weininger J, Malkit M, O'Keane V, **Roddy DW**

Neuroscience Ireland, Dublin 2018

4. The stria medullaris in pain: the first steps towards using diffusion tractography of the SM in the assessment of neuromodulatory pain pathways

Roman E, Levins K, **Roddy DW**

World Institute of Pain, Dublin 2018

5. Subdivisions of the cingulum bundle and changes with age

Grealy A, Murphy E, O'Keane V, **Roddy DW**

The Anatomy Society Summer Meeting, Oxford 2018

6. The human dorsal hippocampal commissure: untangling its connections

Gaughan C, Nasa A, O'Keane V, **Roddy DW**

The Anatomy Society Summer Meeting, Oxford 2018

7. Hippocampal substructural changes over the normal lifespan

Roman E, Adegbeni B, King R, O'Keane V, **Roddy DW**

The Anatomy Society Summer Meeting, Oxford 2018

8. The human thalamocingulate tract - controversy over its anatomical course

Weininger J, Malkit M, O'Keane V, **Roddy DW**

The Anatomy Society Summer Meeting, Oxford 2018

9. Investigating white matter pathology in the dorsal diencephalic conduction system using diffusion tensor tractography: a potential mechanism of chronic pain.

Levins KJ, Elena Roman, **Roddy, DW**

Irish Pain Society ASM, Dublin 2018

10. The dorsal diencephalic conduction system: broadening research into the mechanisms of chronic pain through the creation of a unique diffusion protocol.

Levins KJ, Elena Roman, **Roddy, DW**.

International Association for the Study of Pain (IASP), Boston 2018

11. The amygdala in depression: more than the sum of its parts?

O'Callaghan A, **Roddy DW**

Irish College of Psychiatry, Athlone, June 2017

12. The stria medullaris as a potential biomarker in major depressive disorder

Rooney S, **Roddy DW**

Irish College of Psychiatry, Athlone, June 2017

13. Comparison of atlas-based tractography to manual tractography of the fornix

Roddy DW, Adair A, O'Hanlon E, O'Keane V.

Brain Research Day, Dublin, February 2016

14. ¹H (proton) MRS in Depression – a review of literature and future directions

Roddy DW.

Neuroscience Ireland, Dublin, September 2015

15. Investigating advanced diffusion imaging of the fornix as a potential biomarker

Roddy DW, Adair A, O'Hanlon E, O'Keane V.

Neuroscience Ireland, Dublin, September 2015

APPENDIX 1.2. PAPERS

Roddy DW, Farrell C, Doolin K, Roman E, Tozzi L, Frodl T, O'Keane V, O'Hanlon E: *The Hippocampus in Depression: More than the sum of its parts? Advanced Hippocampal Substructure Segmentation in Depression*. Biological Psychiatry 2018.

Roddy DW, Roman E, Rooney S, Andrews S, Farrell C, Doolin K, Levins KJ, Tozzi L, Tierney P, Barry D, et al: *Awakening Neuropsychiatric Research Into the Stria Medullaris: Development of a Diffusion-Weighted Imaging Tractography Protocol of This Key Limbic Structure*. Frontiers in Neuroanatomy 2018, 12.

Drago T, O'Regan PW, Welaratne I, Rooney S, O'Callaghan A, Malkit M, Roman E, Levins KJ, Alexander L, Barry D, **Roddy DW**: *A comprehensive regional neurochemical theory in depression: a protocol for the systematic review and meta-analysis of ¹H-MRS studies in major depressive disorder*. Systematic reviews 2018, 7:158.

Farrell C, Doolin K, N OL, Jairaj C, **Roddy DW**, Tozzi L, Morris D, Harkin A, Frodl T, Nemoda Z, et al: *DNA methylation differences at the glucocorticoid receptor gene in depression are related to functional alterations in hypothalamic-pituitary-adrenal axis activity and to early life emotional abuse*. Psychiatry Res 2018, 265:341-348.

Doolin K, Allers KA, Pleiner S, Liesener A, Farrell C, Tozzi L, O'Hanlon E, **Roddy DW**, Frodl T, Harkin A, O'Keane V: *Altered tryptophan catabolite concentrations in major depressive disorder and associated changes in hippocampal subfield volumes*. Psychoneuroendocrinology 2018, 95:8-17.

Weininger, J, Roman E, Tierney P, Barry D, Gallagher H, Levins K, O'Keane V, O'Hanlon E, and **Roddy DW**: Papez's forgotten tract: 80 years of unreconciled findings concerning the thalamocingulate tract. *Frontiers in Neuroanatomy*.

ST. VINCENT'S UNIVERSITY HOSPITAL
Brain Medicine Unit

THE STRIA MEDULLARIS IN PAIN

The first steps towards using diffusion tractography of the SM in the assessment of neuromodulatory pain pathways.

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INTRODUCTION

THE STRIA MEDULLARIS

The stria medullaris thalami (SM) is a **discrete limbic white matter tract** that forms the initial part of the dorsal diencephalic conduction system (DDCS), a pathway that allows for basal forebrain structures to influence ascending brainstem pain pathways (1, 2). The DDCS achieves this through the stimulation of the habenula via the SM which leads to the inhibition of serotonergic and dopaminergic systems responsible for **pain modulation** (2). This regulation of monoamines occurs in response to noxious stimulation in humans.

AIMS

This study aims to **develop an anatomically validated and reliable protocol for rendering the stria medullaris** with diffusion weighted imaging (DWI). No study to date has successfully reconstructed the stria medullaris from diffusion imaging due to its small size and high angular path. Up until now the stria medullaris has remained an understudied tract, with limited information with regards to its path and function.

Figure 1. Midsagittal view of the stria medullaris

METHODS

PROTOCOL DEVELOPMENT

Through collaboration with the anatomy department in Trinity College Dublin, **eight dissected human brains** were obtained. A midsagittal dissection through the corpus callosum was made to expose the SM. The anterior landmark of the SM was identified as the anterior commissure, where the SM becomes a discrete tract, and the posterior landmark was identified as the superior most point of the pineal gland.

DIFFUSION WEIGHTED IMAGING

Fifty participants (25 female) were scanned using High Angular Resolution Diffusion Imaging (61 directions, 3 Tesla) as part of the ongoing REDEEM study. Tractography of the whole brain using Constrained Spherical Deconvolution (CSD) modelling of all potential deterministic streamlines was generated. For each fibre, diffusion metrics were obtained and subjected to statistical analysis.

TRACTOGRAPHY

Three dimensional reconstructions of the SM were extracted by applying regions of interest (ROI) gates determined by our anatomical landmark protocol (**figure 2**). Following gate placement, the tracts were visualised. Each tract was checked, examined and cleaned for extraneous streamlines by the rater and a neuroanatomist.

Table 1

	Cadaver	DWI	p-value
Tract Length (mm)	29.273	30.071	0.172
Tract Volume (mm³)	111.661	124.038	0.473

RESULTS

- The SM was visualised consistently in every subject.
- Satisfactory face validity of the protocol** was shown through the comparison of diffusion rendered tracts to anatomical measurements (Table 1).
- Strong inter-rater reliability** was demonstrated ($p=0.88$).
- Analyses between each diffusion metric and age **identified a positive correlation $p=0.038$, $r=0.3$, for Radial Diffusivity (RD)**; axonal packing). No other metric showed a significant correlation with age ($p<0.05$).

CONCLUSION

In this study a reliable protocol for the reconstruction of the functionally important stria medullaris using diffusion weighted imaging was developed and validated. The technique demonstrated excellent inter-rater reliability. **This study is the first to reliably reconstruct the stria medullaris using diffusion MRI**. Identification of the stria medullaris will progress DDCS research and its role in chronic pain states through evaluation of diffusion metrics related to neuronal integrity and myelination states. Accurately localizing the SM through tractography may aid deep brain stimulation of this structure as a treatment for chronic pain.

Figure 2. Reconstructed Stria Medullaris

(1) Buchanan, and J. E. Fraser. 1937. *Buchanan's manual of anatomy including embryology*. Bailliere, Tindall and Cox, London.

(2) Shelton, L., L. Becerra, and D. Borsook. 2012. Unmasking the mysteries of the habenula in pain and analgesia. *Progress in Neurobiology* 96: 208-219.

ATLAS-BASED AND MANUAL DIFFUSION TRACTOGRAPHY OF THE FORNIX A COMPARISON OF TECHNIQUES

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3. Department of Anatomy, Trinity College Dublin, Dublin 2
4. Department of Physiology, School of Medicine, University College Dublin, Dublin 4

Figure 1. Schematic view of fornix (Leighton et al. 1993)

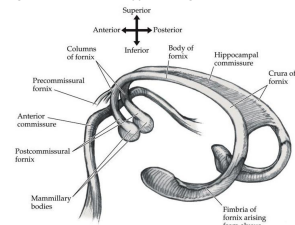
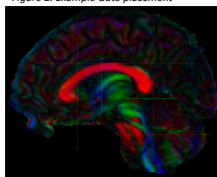
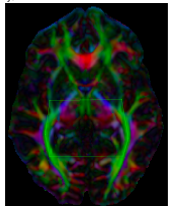


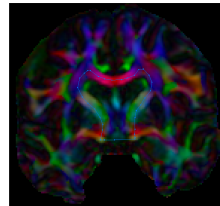
Figure 2. Example Gate placement



Sagittal view showing all gates;
4 NOT gates (red) for extreme of fornix
1 SEED gate (blue) just posterior to column of fornix
1 AND gate (green) to encompass curve of crura of fornix



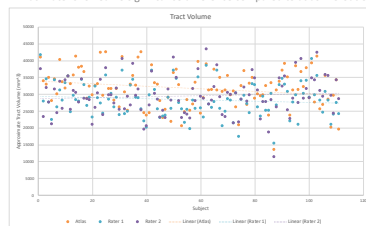
Axial view showing AND gate



Coronal view showing SEED gate

RESULTS

- Inter-rater reliability was strong as no significant differences were found in any of the parameters between the two raters.
- A significant difference in the approximate tract volume was found between both raters and the automated method.
- A significant difference was also found between the automated and manual methods in AD (axial diffusivity; a measure of diffusion along the fibres) and CP (planar anisotropy coefficient).
- No significant difference was found in the following important metrics: FA (fractional anisotropy; a measure of fibre integrity), fibre length (one rater), MD (mean diffusivity) (one rater) and mean angle. The measurements are important as they provide key information on the microstructure of the fornix.
- All other parameters showed no significant difference between the groups.
- Each rater showed no significance difference compared to atlas in 9 out of the 13 parameters.



CONCLUSION

Overall we show good consistency of fornix tractography among the groups – manual (1&2) and automated. (e.g. Figure 3). Some specific subjects showed greater differences (Figure 4). These differences may be due to structural brain differences or placement in the scanner. However, after clean-up, the automated tractography yielded results similar to manual. The most consistent difference between automated and manual tractography was the increased volume of tracts in the automated group. This suggests an over-inclusion of fibres using the automated technique. Over-inclusion is preferable to an under-inclusion which would result in loss of fibres. This over-inclusion can be easily corrected with a simple clean up of extraneous fibres. In conclusion, atlas based automated tractography may be considered a quick and accurate alternative to more time and personnel intensive manual tractography. This could have important clinical implications for the diagnosis of fornix related problems.

THE FORNIX

The fornix is a white matter bundle that is a component of the limbic system. It connects the hippocampus to the mammillary bodies, anterior thalamus and septal forebrain. It is a C-shaped structure in the midline of the brain (Figure 1). The fornix projects posteriorly from the hippocampi as the fimbria which curve superiorly and anteriorly as the crura. The two bilateral crura come together in the midline and continue anteriorly together as the body of the fornix just below the septum pellucidum of the anterior horns of the lateral ventricles. The body continues to arc anteriorly downwards as the columns until it splits into the precommissural and postcommissural fibres at the level of the anterior commissure. Degradation of fornix tracts is associated with various degrees of memory loss ranging from mild cognitive impairment to dementia. The fornix has been shown to be negatively impacted in disorders such as Wernicke encephalopathy, multiple sclerosis, some forms of dementia. In addition, the fornix has been implicated in disorders such as schizophrenia, depression and chronic pain.

AIMS

This study aimed to assess the accuracy and efficiency of semi-automated tractography in comparison to the manual approach. Standard MRI fails to appreciate the complicated anatomy of the fornix. Even though the fornix has been described before in vivo using diffusion tensor imaging, until recently there has been insufficient spatial resolution to allow differentiation of the individual components within the fornix. Semi-automated tractography has been performed in other larger white matter tracts by various authors (Jones et al., 2013), but no study to date has evaluated automated approaches to calculating and reconstructing the fornix from diffusion imaging. Up until now the fornix has been considered too narrow and too convoluted for a semi-automated approach to work. If successful, atlas-based tractography could allow fast and efficient calculation of this underresearched yet significant area and could have an important clinical potential.

METHODS

DIFFUSION TENSOR IMAGING (DTI)

High angular resolution diffusion imaging (HARDI) with 61 diffusion directions was obtained (Field Of View (FOV): 200 x 257 x 126 mm, 60 slices, no gap, spatial resolution: 1.8 x 1.8 x 2.1 mm, TR / TE = 12561 / 59 ms, flip angle = 90°, half k-space acquisition was used (half scan factor = 0.68), SENSE parallel imaging factor = 2.5, b-values = 0, 1200 s/mm², with SPIR fat suppression and dynamic stabilization in an image acquisition time of 15 min 42 s).

DTI DATA POST-PROCESSING

ExploreDTI is a commonly used academic freeware program that is used to process and reconstruct MRI diffusion data. Post-processing and analysis of diffusion images using ExploreDTI involved correction for signal drift, Gibbs ringing, mismatch of orientations, image distortions (eddy current and EPI distortions) and subject motion (including vital rotation of the b-matrix to avoid bias in diffusion estimates). ExploreDTI allows deterministic fiber tractography, clustering of fiber tracts and data quality assessment tools as well as Constrained Spherical Deconvolution (CSD)-based tractography. CSD enables a more accurate reconstruction of local fiber orientation in crossing fiber regions compared to deterministic or probabilistic methods. For each fibre, variables like number and volumes of tracts, fractional anisotropy (FA), axial (longitudinal, L1), radial (RD) and mean diffusivity (MD) were obtained and were subjected to statistical analysis.

FOR CSD-DTI-TRACTOGRAPHY

All data was transformed into MNI space. Seed point resolution was 2 x 2 x 2 mm with a seed FA threshold of 0.2. Deterministic tractography was applied with ExploreDTI. First, whole brain tractography was conducted in each subject using a linear interpolation. For all subjects, gates were placed in a standardized fashion. Jones et al provided an initial reference for placement of fornix gates. We modified and refined this technique after multiple iterations to what we found to provide better fornix tracts. See figure 2 for gate placements. Gates were placed on all subjects by two trained raters (DR & SA). Following gate placement tracts were reconstructed from whole brain tractography. The robustness of the reconstructed fornices were checked by both raters and a separate trained researcher (EOH).

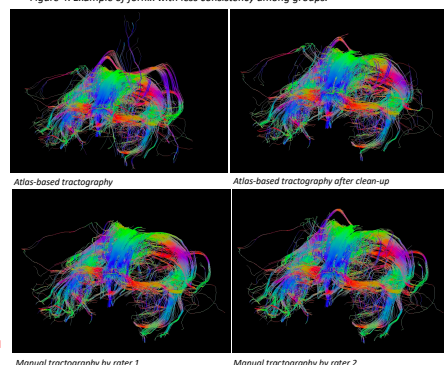
ATLAS-BASED TRACTOGRAPHY

In atlas-based tractography, the same set of standard gates were drawn onto the brain of a random average subject which acted as the template. Automated placement of gates was calculated with reference to the template subject.

	Rater 1 vs Rater 2	Rater 1 vs Atlas	Rater 2 vs Atlas
Number of tracts	NS	NS	NS
Mean tract length	NS	NS	0.044
Approx. tract volume	NS	3.3×10^{-6}	0.003
Mean FA	NS	NS	NS
Mean MD	NS	0.04	NS
Mean AD	NS	0.006	0.006
Mean λ_1	NS	NS	NS
Mean λ_2	NS	NS	NS
Mean RD	NS	NS	NS
Mean CL	NS	NS	NS
Mean CP	NS	1.9×10^{-9}	0.0002
Mean CS	NS	NS	NS
Mean angle	NS	NS	NS

Table 1. NS= not significant, FA=fractional anisotropy, MD= mean diffusivity, AD= axial diffusivity, RD= radial diffusivity, CL= linear anisotropy coefficient, CP= planar anisotropy coefficient, CS= spherical anisotropy coefficient. All values shown are the p-values; significance for p<0.05.

Figure 4. Example of fornix with less consistency among groups.



The Human Dorsal Hippocampal Commissure: Using high resolution tractography to delineate the afferents traversing this midline structure

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Introduction

Dorsal Hippocampal Commissure

Lying on the lyra of the fornix, the dorsal hippocampal commissure (DHC) allows communication between the left and right hippocampal and parahippocampal limbic systems. Although a lot of work has been done on the connections of the DHC in animal studies, there is a paucity of research concerning this structure in humans.

Purpose

The purpose of this investigation was to use combined diffusion tensor and structural neuroimaging to determine which hippocampal and parahippocampal substructures comprise the fibres of the dorsal hippocampal commissure. We have grouped substructures based on anatomical proximity here to allow for greater accuracy. The regions examined are the hippocampal formation (HC), Parahippocampal Gyrus (PG), temporal pole (TP) and Amygdala (AMY).

Method

Diffusion weighted imaging

Forty-three healthy participants (18 Males (aged 16-59): 25 Female (aged 18-61), and thirty-one depressed participants 9 Male (aged 17-45): 22 Female (aged 17-64) were scanned using High Angular Resolution Diffusion Imaging (HARDI) in a 3 Tesla scanner using 61 directions as part of the ongoing REDEEM study.

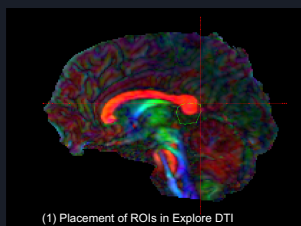
DWI Pre-processing

ExploreDTI, a diffusion magnetic imaging toolbox, was utilised for complete pre-processing, modelling and computation of diffusion data. This allows for the generation of tractography of the whole brain using Constrained Spherical Deconvolution (CSD) modelling of all potential deterministic streamlines throughout the entire brain.

Tractography

Stage 1: to isolate the hippocampal commissure

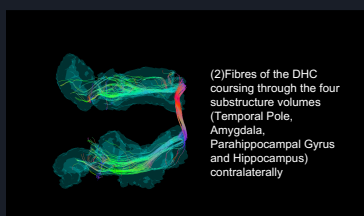
- Boolean logic ROIs were strategically placed as shown below(1) using a strictly implemented protocol.



(1) Placement of ROIs in Explore DTI

Stage 2: to clean the commissure

- NOT gates were used to remove extraneous fibres (e.g. fornix, inferior fronto-occipital fasciculus, optic tract etc.) which appear either due to over-accommodation by the gates from our stage 1 protocol or by miscalculation due to the close proximity with the DHC.
- The left and right combined masks (TP, AMY, HC, PG), which were generated using Freesurfer 6.0, were used as AND gates to remove fibres that by miscalculation, either were not commissural or did not join the two combined structures.

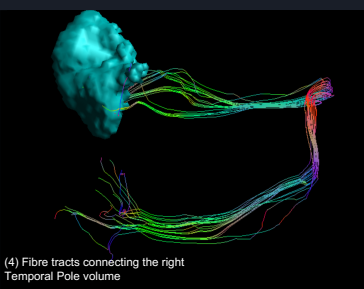


Stage 3: to isolate the fibres terminating in each subfield/region

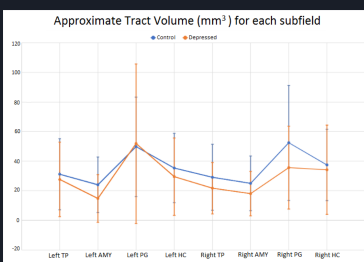
- A logical sequential method involving freesurfer generated subfield masks was generated using anatomical knowledge of the amygdala, hippocampal formation, parahippocampal gyrus, and the temporal pole.
- The sequential method involves the determination of the fibres that terminate in each subfield specifically, using boolean logic, applied on the basis of proximity to the centre of the commissural fibre path.

(3) A DHC with freesurfer 6.0 generated masks for structure on the left:

TP (red)
Amy (green)
PG (blue)
HC (magenta)



(4) Fibre tracts connecting the right Temporal Pole volume



Results

The majority of fibres connect to the parahippocampal gyrus while a minority connect the amygdala. However neither group is statistically significantly different from others, hence though the parahippocampal region may contribute a small majority of the commissure due to its greater standard deviation, overall the spread of volume remains almost equal.

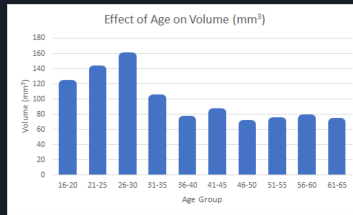
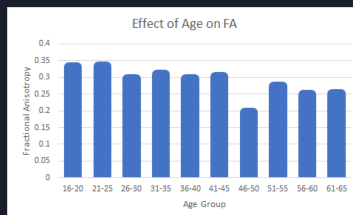
This strongly indicates that the DHC is neither purely hippocampal nor parahippocampal in origin.

There are no significant differences between the controlled and depressed participants for fibres connecting to any region when considering either microscopic or macroscopic metrics.

When corrected for gender and eTIV there is a negative correlation between FA (fractional anisotropy) and age, and volume and age for the commissure, peaking in the mid to late twenties for both values. However FA appears to peak at a younger age but decline at a slower rate than volume.

Commissural volume and FA decrease over the life-span.

	Mean of approximate tract volume (mm³)		SD of approximate tract volume (mm³)	
	Control	Depressed	Control	Depressed
Whole Commissure	79.50	80.49	20.02	61.49
Left temporal pole	27.07	27.87	10.00	26.79
Left amygdala	12.07	14.24	4.70	16.07
Left parahippocampal gyrus	30.77	31.78	10.00	33.84
Left hippocampus	10.00	28.44	10.00	36.04
Right temporal pole	26.00	21.44	10.00	17.04
Right amygdala	14.00	14	10.00	10.00
Right parahippocampal gyrus	31.34	35.04	10.00	27.04
Right hippocampus	27.16	34.1	10.00	33.07



Conclusion

Despite its name, the human DHC is not entirely hippocampal in origin. In fact, the majority of fibres making up the DHC arise from regions other than the hippocampus, including the amygdala. This is the first study to examine the locations of origin and termination of the human DHC using tractography and neuroimaging methods.

This study also shows that the DHC is not affected by depression despite its connections to regions such as the amygdala and the left hippocampus proper, which have been shown to be affected.

Papez's forgotten tract: 80 years of unreconciled findings concerning the thalamocingulate tract

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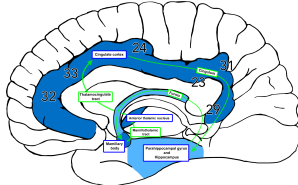
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INTRODUCTION

The Thalamocingulate Tract

The thalamocingulate tract is a white matter tract that extends from the anterior thalamic nucleus into the cingulum bundle. Papez¹ (1937) proposed that the thalamocingulate tract as part of the Papez circuit which is believed to play a significant role in emotional regulation.

Figure 1: The Papez circuit



This literature review seeks to clarify the disagreements in the literature about the exact nature of this tract after it leaves the anterior thalamic nucleus and enters the either the posterior or anterior cingulate region.

Prior research indicates that the anterior thalamic nuclei (ATN), which includes the Anteromedial (AM), Anterodorsal (AD) nuclei, are involved in producing a 'selective alerting response' that upregulates category-specific memories while also inhibiting retrieval of other categories of memories⁴.

The posterior cingulate cortex (PCC) has been implicated in the functioning of the default mode network, de-activating when attention is externally directed, while the anterior cingulate gyrus (ACC) through its connections with the frontal and parietal lobes and limbic structures contributes to a diversity of intellectual, emotional, and motor functions the disruption of which as been noted to cause apathy and uncontrolled vivid daydreaming⁴.

How these regions interface may have significant implications for the understanding of the functioning of the Papez circuit.

FINDINGS

Study	Year	Method	Specific Course	Classification of cingular connection entry
Shah, et al	2012	Dissection, human subject	ATN -> anterior limb of internal capsule -> transverse corpus callosum -> undersurface of Area 24 of cingulum.	ACC
Jang and Yeo	2013	Probabilistic fibre tracking using diffusion tensor tractography (DTT), human subjects	ATN -> anterior limb of internal capsule -> Area 24 -> posteriorly through cingulum.	ACC
Wei et al.	2017	Seed based diffusion tensor imaging (DTI), human subjects	ATN -> anterior limb of the internal capsule -> loops laterally around caudate nucleus -> fans into areas 23 and 29 (RSC) cingulum, few fibres to 24.	ACC
Vogt et al.	1987	Horseradish peroxidase (HRP), rhesus monkey	AM nucleus sends fibres into posterior cingulate cortex primarily 23 while AV, AD, LD (LD not ATN) sends fibres Area 29 of the retrosplenial cortex (RSC)	PCC and RSC
Matsuoka	1986	HRP, cat	Fibres project to entirety of cingulum with greatest density in area 24	ACC
Baleydier and Mauguier	1980	HRP, multiple species of monkey	AM nucleus -> area 24 primarily, 23 to a lesser extent AV, AD, LD -> area 23	Full cingulum
Musil and Olson	1988	Retrograde tracing, Cat	AM -> area 24 (ACC homologue) area 23 (PCC homologue) -> AM (afferents and efferents differentiated)	Full cingulum
Finch, Derian, and Babb	1984	Retrograde tracing, rat	AD -> area 23 (PCC homologue) ATN -> PCC area 29 of RSC	PCC and RSC
Mufson and Pandya	1984	Radiolabelled amino acids, rhesus monkey	ATN -> rostral beneath caudate nucleus -> anterior limb of internal capsule -> ventral sector of cingulum, spans all areas	Full cingulum
Shibata and Naito	2005	PHA-L anterograde tracing, rat	Rostral AM sends fibres to rostral ACC, caudal AM sends fibres to caudal ACC, laminar distribution	ACC
Niimi	1978	Lesion-induced degeneration tracing, cat	ATN -> internal capsule -> lateral margin of caudate nucleus -> cingulum Significant overlap in fibres from respective thalamic nuclei. AM -> ACC AD -> ACC and middle cingulum AV -> middle cingulum and PCC	Full cingulum
Clark and Boggan	1933	Lesion-induced degradation tracing, cat and rat	none described	Full cingulum

DISCUSSION OF POTENTIAL EXPLANATIONS OF DIVERGENT FINDINGS

There were a number of methodological concerns which may be relevant to explain the lack of consensus on the anatomical course of this tract.

A) Tracing studies that followed radiations of the individual thalamic nuclei (AV, AM, AD) found that other thalamic nuclei such as the laterodorsal (LD) thalamic nuclei pass fibres to the cingulum and associated limbic and pre-frontal regions. This may be influencing the imaging studies which utilize masks and gates that do not isolate anterior thalamic radiations from other thalamic radiations. Future studies may require more granular differentiation of thalamic nuclei afferents.

B) We found that studies that found identified connections with the posterior cingulate did not always differentiate fibres that entered the posterior cingulate and the retrosplenial cortex. This may be leading to an over-classification of thalamocingulate tract as terminating in the posterior cingulate cortex relative to the

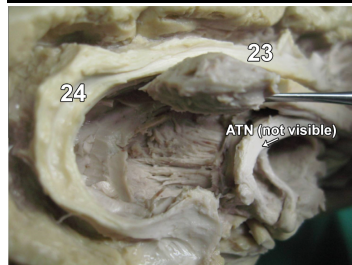
anterior portion.

C) Several studies³ noted significant anatomical variation among subjects. Given the low number of subjects in many of these studies, this may contribute to divergent results.

D) DTI based techniques alone are incapable of distinguishing between afferent and efferent neuronal connections. Establishing directionality of tracts reliably requires morphological information. Such a differentiation may confirm the findings of certain studies indicating that multiple distinct regions of the tract include afferent and efferent connections, complicating the understanding of the course of the tract.

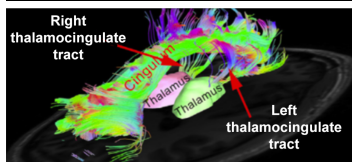
E) Many prior studies on non-human indicate that the structures in rodents, non-human mammals, and humans are homologous. If further human research diverges from prior animal studies, this assumption may be put into question.

Figure 2: Dissection displaying connection to ACC



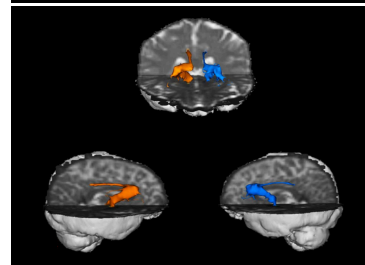
Shah et al. 2012 dissection shows radiations originating from the thalamus under a lifted caudate head extending rostrally to the ventral cingulum surface.

Figure 3: Seed-based DTI displaying connection to PCC



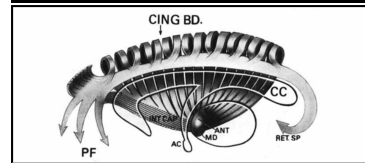
Wei et al using DTI fibre tracking isolated fibres turning into area 23 as well as into the RSC.

Figure 4: Probabilistic fibre tracking displacing connection to ACC



Jang and Yeo using probabilistic fibre tracking using ATN, the anterior limb of internal capsule and the cingulate gyrus as regions of interest show fibres extending rostrally into cingulum bundle

Figure 5: Diagram of findings displaying ATN connection to entire rhesus cingulum



Mufson and Pandya 1984 diagram of radiolabelled amino acid study. Fibres fan out through extent of cingulum bundle.

LITERATURE REVIEW METHODOLOGY

This literature review was conducted using PubMed/MEDLINE, Google Scholar, EMBASE, The Cochrane Library, OVID and PsycINFO and resources within the Anatomy Department Trinity College Dublin. Studies were identified with the keywords 'thalamocingulate tract', 'anterior thalamic projections', 'thalamocortical radiations', 'anterior thalamic nuclei to cingulate cortex'. Information collected pertained to the methodology, course, and termination of the thalamocingulate fiber tracts.

OVERVIEW OF FINDINGS

The results from the 12 studies reviewed were found to strongly conflict one another. These findings can be broadly sorted into three categories.

- 1) One group consists of four studies which found the connections passing medially and rostrally after leaving the internal capsule and primarily entering the cingulum in the anterior portion of the cingulate cortex.
- 2) A second group of three studies found that fibres passing through the internal capsule made a hard caudal turn, entering the cingulum through the posterior cingulate cortex.
- 3) A third group of five studies did not find a significant preponderance of fibres in either component of the cingulate cortex, but rather found that the fibres fan out to enter throughout the extent of the cingulum.

There was no apparent divergence in results between the examined human studies versus non-human studies, nor in the specific technique used in these studies. However the number of studies conducted was not high enough to conclusively demonstrate these are not a factor biasing results.

Diffusion Based Tractography Changes of the Cingulum Bundle

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INTRODUCTION

The cingulum bundle is a limbic association pathway which communicates with frontal, parietal and medial temporal cortices. The cingulum forms a near-complete circle from the orbital cortices, along the dorsal surface of the corpus callosum and down the temporal lobe towards the pole (1). It is a key structure in the Papez circuit and as such is essential for the development of cognitive and emotional skills through adolescence and beyond. Previous studies suggest that the cingulum reaches maturation during the mid-twenties onwards. However, few studies focusing on this structure have coherently determined its partitions as well as related vicissitudes throughout life's cycle. Research has instead been centred on associations and functions of the cingulum. Therefore, the goal of this investigation was to use diffusion tensor imaging to separate the human cingulum bundle into subdivisions based on surrounding anatomical structures and to determine structural alterations of these regions during maturation into adulthood.

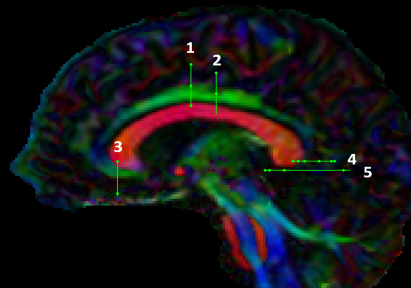


Figure 1. ROI gate placement. A total of 5 ROIs were placed following the Jones et al. 2013 protocol.

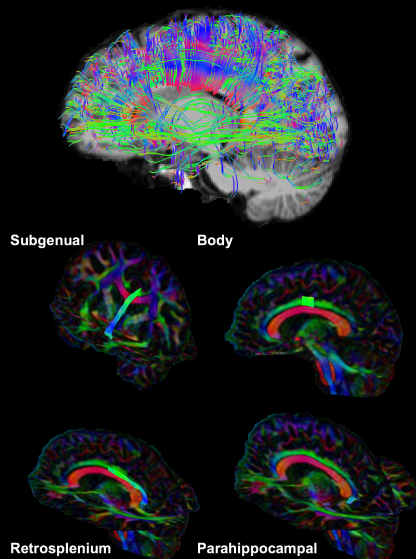


Figure 2. The first image shows the rendered cingulum after ROI gate placement and pre-segmentation. The subsequent image depicts subgenual, body, retrosplenium and parahippocampal sections of the cingulum after segmentation and cleaning.

METHODS

A total of 50 healthy (25 female) participants aged 15 - 40 years, were scanned using a High Angular Radial Diffusion Imaging (HARDI) protocol. Participants were recruited as part of the REDEEM group in Trinity College Dublin. Following constrained spherical deconvolution whole brain tractography, the cingulum bundle was reconstructed. The cingulum was divided into anatomic-functional segments (subgenual, body, retrosplenium and parahippocampal) by two independent raters. A total of 5 region of interests (ROIs) were utilised to reconstruct these different divisions. ROIs were placed following the previously described protocol by Jones et al 2013 (2). Following ROI placement, the regions were segmented using the ExploreDTI tool and tracts inconsistent with cingula anatomy were removed. Cingula dimensions and diffusion metrics (measures of structural integrity) were then calculated and compared between gender and age. Statistical analysis was done through SPSS24. Ethical approval for this investigation came under the remit of the REDEEM study at Trinity College Dublin.

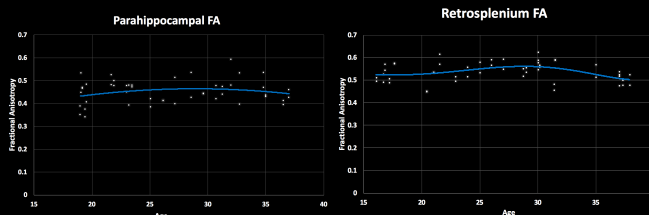


Figure 3. FA of the parahippocampal cingulum peaked in females between 25 to 30 years. Figure 4. Retrosplenium FA increased between 25 to 30 years in males.

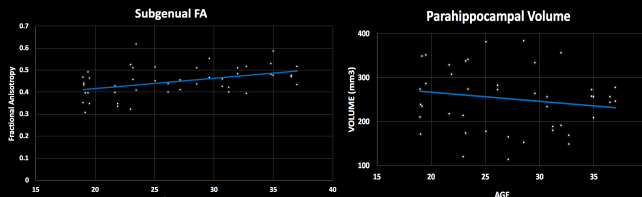


Figure 5. Subgenual FA increased continuously over time.

Figure 6. Parahippocampal volume decreased in females consistently over time.

RESULTS

- Excellent inter-rater reliability in tract reconstruction and segmentation was demonstrated.
- Anatomically specific variations in maturation and stability occurred along the cingulum tract.
- Volume decreases were most notable in the parahippocampal area in females over time.
- Fractional anisotropy was found to be significantly lower between 15-20 years and 35-40 years in the parahippocampal and retrosplenium suggesting maturation of these cingulum regions in the mid- to late- twenties, followed by decline. This is consistent with previous literature.
- Interestingly, there was a continuous increase in fractional anisotropy in the subgenual region in females only. This region is significant as it is involved in cognitive processing and emotional regulation. Further studies need to be conducted to investigate the relationship between the subgenual and emotional maturation.

CONCLUSION

These findings strongly suggest that the cingulum does not mature consistently throughout its structure. The data is indicative of parahippocampal and retrosplenial maturation in the late twenties, while the subgenual area has been seen as more likely to mature later in life. An understanding of the normal variation in maturation of the cingulum may prove vital in the assimilation of underlying pathological abnormalities in neurodevelopmental disorders as well as underlying the difficulties associated with adolescent emotional control.

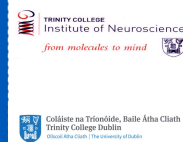
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The Amygdala in Depression. More than the sum of its parts?

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Background

Amygdala dysfunction has been implicated in major depressive disorder, with numerous studies consistently demonstrating a hyperactive amygdala in depressed adults. This is postulated to be a result of higher amygdala metabolism and blood flow. However, previous studies have failed to demonstrate consistent differences in amygdala volume in depressed patients versus controls. Some studies have shown currently depressed subjects to have larger amygdala volumes bilaterally compared with both recovered patients and healthy controls¹. Some studies have shown a decrease in amygdala volume on the left side in depressed patients², while others have shown increased amygdala volumes in both hemispheres as compared to healthy control subjects³, and others have shown bilateral reductions in specific areas. Discrepancies between studies may be due to differences in amygdala boundary definitions.

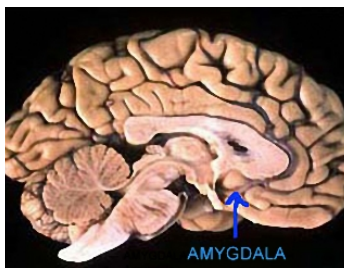


Fig. 1: Position of amygdala

Objectives/Aims

To apply advanced MRI based automated segmentation to a well-characterised group of depressed patients and controls to investigate amygdala subfield volume. Comparison in amygdala volume between first presentation depression, recurrent depression and controls.

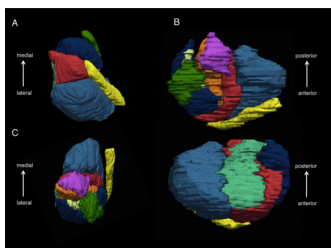


Fig. 2: 3D rendering of manual segmentation based on MRI in one ex vivo case

Methods

MRI scans were compared between 80 secondary care patients meeting diagnostic criteria for major depressive disorder and 83 healthy controls as part of the REDEEM (Research in Depression: Endocrinology, Epigenetics and neuroimaging) study in Trinity College. Patients were subdivided into first presentation depression (43) and recurrent depression (37) groups. High resolution T1 and T2 – FLAIR weighted MRI brain scans were examined using the automated amygdala substructure volumetric module in Freesurfer version 6.0. Between-group volumetric assessment of substructures was performed.

Results

Nine nuclei were identified in each amygdala hemisphere. These were divided into three composite groups: **Laterobasal** (Lateral, Basal, Accessory Basal and Paralaminar), **Centromedial** (Central and Medial), **Superficial** (Cortical, Anterior Amygdala Area and Corticoamygdaloid transition Area). Statistically significant differences were found in the total depression cohort on the right side in the centromedial area ($p=0.024$) and in the recurrent depression cohort in the same area ($p=0.003$). No statistically significant changes were found in the composite areas on the left. No significant changes were found in the whole amygdala volume between right and left. This is in contrast to previous studies which have shown the left side to have decreased volume². On the left side, there were statistically significant differences in inflammatory and cortisol-related biomarkers. There was an inverse correlation between CRP and amygdala volume. There was a similar inverse correlation between cortisol response and amygdala volume, again on the left side only.

Biomarker differences								
	Left			Right				
	Lateral Nucleus	Basal Nucleus	Whole Amygdala	Lateral Nucleus	Basal Nucleus	Whole Amygdala		
IL6 ELISA	NS	NS	NS	NS	NS	NS		
IL10 ELISA	NS	NS	NS	NS	NS	NS		
CRP ELISA	NS	-ve (0.001)	-ve (0.0002)	NS	NS	NS		
AUCb	NS	NS	NS	NS	NS	NS		
AUCg	-ve (0.004)	-ve (0.029)	-ve (0.004)	NS	NS	NS		
AUCi	-ve (0.00006)	-ve (0.00005)	-ve (0.002)	NS	NS	NS		
RT	-ve (0.001)	-ve (0.0004)	-ve (0.004)	NS	NS	NS		

Fig. 3: Biomarker Differences

Volume differences						
	Left			Right		
	Con ¹ vs AD ²	Con ¹ vs FPD ³	Con ¹ vs RD ⁴	Con ¹ vs AD ²	Con ¹ vs FPD ³	Con ¹ vs RD ⁴
Whole Amygdala Volume	0.77	0.74	0.79	0.25	0.81	0.13
Laterobasal	0.76	0.73	0.78	0.29	0.78	0.2
Centromedial	0.52	0.21	0.75	0.024	0.33	0.003
Superficial	0.81	0.45	0.91	0.68	0.61	0.27

Fig. 4: Volume Differences:
¹Controls ²All Depressed ³First Presentation Depression, ⁴Recurrent Depression

Conclusion

This study identified right-sided differences across the centromedial composite group of amygdala nuclei in major depressive disorder consistent with its key role in emotional processing. In general these changes were more pronounced in recurrent depression than first presentation depression. The biomarker differences were statistically significant in the left laterobasal area. On the right there is a correlation with physical size in the output area while on the left there is a correlation with inflammatory markers in the input area. **This suggests a left/right asymmetry across both input/output nuclei and size/inflammatory response in the amygdala.** Also amygdala volume may decrease with prolonged disorder duration and emphasizes further the need for early therapeutic interventions in major depressive disorder to halt the progression of amygdala pathology. Prospective investigations to investigate amygdala changes during the course of depression may also prove beneficial.

Acknowledgements

- This study was funded by the Health Research Board Ireland.
- Ethical approval: Tallaght/St. James' REC

References

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- Enlargement of the Amygdala in Patients with a First Episode of Major Depression, Frodl et al, 2002. Larger Amygdala Volumes in First Depressive Episode as Compared to Recurrent Major Depression and Healthy Control Subjects, Frodl et al, 2003.
- Fig. 1: Sheep Brain Digital Dissection, www.thinglink.com/scene/74530548351848610, accessed 9/04/2018
- Fig. 2: High-resolution magnetic resonance imaging reveals nuclei of the human amygdala: manual segmentation to automatic atlas, Fig. 4: Saygin 2017.

Further information: aoifekocallaghan@gmail.com

APPENDIX 2.1. PATIENT INFORMATION SHEET AND CONSENT



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INFORMATION SHEET

Title of the Project:

The REDEEM Study **Research in Depression: Endocrinology, Epigenetics and neuroiMaging**

LEADER OF STUDY

The Principal Investigators in The REDEEM Study:

PROFESSOR VERONICA O'KEANE,
Trinity College Dublin & Tallaght Psychiatry Services

This project is funded by the Health Research Board (HRB), the EU (Marie Curie Fellowship) and the Meath Foundation.

SUMMARY OF STUDY

Depression is a complicated disorder. Your genes, personality type and life stress all contribute to causing depression. Some people are more likely to become depressed because they may have a strong family history of depression: for example, if both parents have suffered from depression. Most people, however, become depressed because of a combination of things. For example, they may be stressed because of things that are happening, and/or they may be a sensitive personality type, and/or they may have had a difficult childhood. Often people who become depressed do not have a person to confide in that they can trust emotionally.

In the REDEEM Study we are interested in finding out how the brain and body changes when someone becomes depressed. We want to see what happens in the body's stress and immune systems and in brain structure and function when someone is depressed. We want to then look at any changes in these systems over a period of time (approximately one and a half years).

WHY HAVE I BEEN CHOSEN?

You have been chosen because you have either major depression or are a healthy person who can be a "control" for (someone to compare with) a depressed person. We are looking at specific groups of depressed people: those who are experiencing their first episode of depression; those who have had previous episodes of depression and those who were tested by our group five years ago. For those who are experiencing their first episode of depression, we would like you to repeat some of the tests in six months, and some at one year following this.



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DO I HAVE TO TAKE PART?

No. It is completely your decision whether you take part or not. If you want to get involved with the study, this Information Sheet will be explained in detail to you. You will be asked to sign the Consent Form if you decide to participate. You may withdraw from the study at any time you wish and without giving a reason. This will not affect the care you receive from your doctor.

WHAT WILL HAPPEN IF I AGREE TO TAKE PART IN THIS STUDY?

If you decide to participate, we will ask you to do an interview and fill out some questionnaires to look at your symptoms and experiences. This will take about 40 minutes.

During this appointment a doctor or Research Worker will arrange the following tests

- (1) collection of blood, saliva and urine samples
- (2) a brain scan

(1) Collection of blood, saliva and urine samples

We wish to examine the levels of the stress hormone cortisol. The stress hormone cortisol is the main stress hormone in our bodies. Cortisol affects our brain and mind and is the reason why we have difficulty sleeping, concentrating and why we feel anxious when stressed or depressed. If we are very stressed, our levels of cortisol are high. People suffering from depression have high levels of cortisol throughout the day.

We want to measure how cortisol levels increase following waking up and in the evening. Depressed people tend to secrete high cortisol levels when they awake and to have high cortisol levels in the evening. We want to see if the secretion of cortisol goes down following treatment for depression.

What we will ask you to do:

Saliva: we will give you small tubes in which to collect samples of saliva. The instructions are written down and we will go through this with you.

Urine: we will ask you to collect a small sample of urine in a plastic container that we will supply. This collection will allow us to measure the total amount of cortisol that you secrete.

Our research worker will arrange collection of the samples.

Blood: we will take some blood samples from you: in total about 50ml in five blood tubes. The blood samples will be taken by one of the research workers either when you fill out the questionnaires or when you go to Trinity College to have your scan. Your blood samples will be anonymous (will only have a number, not your name).

Most of the blood will be analysed in the Trinity College Institute of Neuroscience Dublin by our research team. We want to measure cortisol, stress related genes and immune substances in the blood.

Some of the samples will be sent anonymously to our research partners in McGill University Montreal, Canada, University Hospital of South Manchester, as well as Trinity Biomedical Sciences Institute to see how your genes are working. At a later stage these results may form part of a collaborative study with researchers in Ireland and abroad.



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(2) Brain scan

A brain scan, or neuroimage, allows us to see the different structures in the brain, giving a picture of the different parts of the brain. The type of scan that we will use is an MRI scan. It is a safe procedure that usually takes about 60 minutes.

We will do the MRI scan in Trinity College, in the Lloyd Building. The MRI machine uses a magnetic field to take pictures of the brain. Before you enter the room where the machine is located, you will have to remove all the metallic things you may be wearing such as bracelets, earrings, watch, or keys. You will be asked to lie on a couch for about 60 minutes while the MRI machine gathers information.

While you are lying on the couch, you will be able to speak with the operator of the scanner at any time. In addition, you will have a button in your hand that you can use to stop the measurement at any time.

We may ask you some very short questions about your mood/thoughts after the scan.

WHAT WILL BE THE POSSIBLE BENEFITS OF TAKING PART?

It is unlikely that any of the information from the "biological" part of the study will tell you or us much about you or what treatments might be good for you. If there is some unexpected finding in your blood tests or in your scan, such as an abnormal blood vessel in the brain, we will provide whatever care you may need.

No individual genetic or non-genetic result will be available from the study, to you or to anyone else, and this study does not involve screening for genetic diseases.

If you wish, we will keep you informed of the progress of the study in general.

We can also furnish you with non-clinical images of your brain if you wish.

WHAT ARE THE POSSIBLE DISADVANTAGES OF TAKING PART?

The risk for the taking of the blood sample is the same as for any standard blood-taking procedure and is very safe. There is the usual minor discomfort/pain with the procedure but it will be in your arm where the puncture is and will be present for a short while only. All our researchers are very experienced in this technique.

The MRI machine uses a strong magnet to make images of the brain. You will be asked to lie on a long narrow couch for about 60 minutes while the machine gathers data. During this time you will be exposed to a magnetic field that you will not feel. You will, however, hear repetitive tapping noises that arise from the scanner around your body. We will provide earplugs and headphones that you will be required to wear so that it is not loud. The space within the large magnet in which you lie is somewhat narrow, although we have taken many steps to relieve the "claustrophobic" feeling.

There are no known significant risks with this procedure since the main magnetic field at the strengths used are felt to be without harm. MRI in pregnant women will be avoided in the



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present study. People who cannot be scanned using MRI include persons who have piercings that cannot be removed, cardiac pacemaker or a certain type of metallic clip in their body (i.e., an aneurysm clip in the brain); persons who have worked with metal or had a piece of metal removed from the eye(s); or persons who have shrapnel, bullets, or buckshot in their body.

There is a risk of heating from the imaging coils, and/or the cables from monitoring devices that record physiologic processes such as heart beats per minute. Please report any heating sensation immediately. You may have the scan stopped at any time if this occurs.

There is a possibility that you will experience a localized twitching sensation due to the magnetic field changes during the scan. This is not unexpected and shouldn't be painful. However, you may have the scan stopped at any time if this occurs.

While participating in this study, you should not take part in any other research project without approval from all of the investigators. This is to protect you from possible injury arising from such things as extra blood drawing, effects of research drugs, or similar hazards.

WILL MY TAKING PART BE CONFIDENTIAL?

Yes. All information, which is collected about you, during the course of the research, (including scanning, blood, saliva, urine and questionnaire data) will be kept strictly confidential and anonymous. Your name will not be attached to any information about you.

You will only be identified by a number in the REDEEM Study. The key to this number code and any personal information will be kept confidentially by Professor Veronica O'Keane in Trinity College Dublin.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

The results will be published in scientific journals and presented at conferences. We hope to present the results of the study in the general media and public venues as well.

LEGAL ISSUES:

The doctors involved in this study are covered by standard medical malpractice insurance.

PERMISSION:

This study has been approved by the St. James's and Tallaght Hospital Ethics Committee



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FURTHER INFORMATION:

If you would like to obtain further information about the nature of the study you can do so by contacting:

Prof. Veronica O'Keane

Department of Psychiatry
Tallaght Psychiatry Services
Sheaf House
Belgard Square North
Tallaght
Dublin 24
Ireland
Tel: 01 463 5200

Supplemental contact and study information may be found at our website:

redeemtc.d.ie

Thank you for your help with this project.



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PARTICIPANT STEP-BY-STEP INSTRUCTIONS FOR SALIVA COLLECTION

- The purpose of the saliva collection is to measure the changes in stress hormones throughout the day.
- On the **first day**, the saliva will be collected by putting the absorbent sponge **under your tongue** for two minutes until wet, and then inserting it into the top compartment of the blue tubes provided.
- The tubes will be labeled with **time points** corresponding to each collection after waking.
- You will have **5 saliva collections** in total. You should put them in the plastic bag provided.
- Please **bring the tubes with you to your brain scan at Trinity**, which should be one day after your collection period. Or we will arrange to collect them as convenient. The samples will remain fresh for a few days.

The saliva tubes will be labeled for the specific times below. Please collect saliva at each of these times after waking on Day 1.

1. 0 minutes
2. 30 minutes (after waking)
3. 60 minutes (after waking)
4. 12 hours (after waking, in the pm)
5. 12.5 hours (after waking, in the pm)

For example, if you wake up at 7.00 am, you will be collecting samples at:

1. 7.00 am (approximately)
2. 7.30 am
3. 8.00 am
4. 7.00 pm
5. 7.30 pm

How to take samples:

Please do not brush your teeth prior to collecting the morning samples and rinse your mouth out 5 min before taking a sample (please make sure that you only collect saliva and not water from rinsing your mouth out!). If you forget to take a sample, just move on to the next time point.

If you have any questions, please contact Dr. Darren Roddy

Tel: 0866087927

Email: dwroddy@tcd.ie



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The Lloyd Institute is located at the back of the Trinity College Campus, indicated with the red star.
Contact **Dr. Darren Roddy** if you have any questions about how to get to the Lloyd Institute for your MRI scan.

Tel: 0866087927
Email: dwroddy@tcd.ie



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Consent Form

Title of research study:

The REDEEM Study

Research in Depression: Endocrinology, Epigenetics and neuroimaging

Principal Investigator: Professor Veronica O'Keane, Trinity College Dublin & Tallaght Psychiatry Services &

This study and this consent form have been explained to me. My doctor has answered all my questions to my satisfaction. I believe I understand what will happen if I agree to be part of this study.

I have read, or had read to me, this consent form. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study. I have received a copy of this agreement

PLEASE TICK YOUR RESPONSE IN THE APPROPRIATE BOX

- I have read and understood the attached Participant Information Leaflet
Yes ☐ No ☐
- I have had the opportunity to ask questions and to discuss the study
Yes ☐ No ☐
- I have received satisfactory answers to all my questions
Yes ☐ No ☐
- I understand that I am free to withdraw from the study at any time without giving a reason and without this affecting my future medical care
Yes ☐ No ☐

PARTICIPANT'S NAME:

PARTICIPANT'S SIGNATURE: _____ **Date:** _____

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits and risks of this research study. I have offered to answer any questions and fully answered any questions asked. I believe that the participant understands my explanation and has freely given informed consent.

Physician's/Scientist's NAME:

Physician's/Scientist's SIGNATURE: _____ **Date:** _____



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The REDEEM Study

Research in Depression: Endocrinology, Epigenetics and neuroiMaging

For participant aged less than 18 years

If you are under 18 years old the signature of your parent or guardian must be obtained

NAME OF PARENT or GUARDIAN:

SIGNATURE: _____ DATE: _____

RELATION TO PARTICIPANT: _____

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to, this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

Physician's/Scientist's signature:

Date:



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The REDEEM Study

Research in Depression: Endocrinology, Epigenetics and neuroimaging

Consent Form for Biobank

I am (a) 18 years of age or more, or (b) I am a parent of a minor aged between 16 and 18 years.

I consent to the taking of a blood sample for storage in the Biobank in the Trinity Centre for Health Sciences, St James's Hospital.

Signed _____ Date _____

Witnessed by _____ Date _____

APPENDIX 2.2. DATA CONSENT FORM MRI



GENERAL MRI DATA CONSENT FORM

Trinity College Institute of Neuroscience, (TCIN) is performing research, utilising an MRI scanner at Trinity College, Dublin 2. These research scans, although not full clinical scans, will be read by a radiologist.

In the unlikely event of an irregularity being found, the radiologist, [Dr William Torreggiani of The Adelaide and Meath Hospital Incorporating the National Children's Hospital (AMNCH), Tallaght] will inform the participants GP, that a proper clinical scan may be required to determine whether or not an irregularity is of clinical significance.

To enable us to perform the research scans the participant agrees to give consent/ permission for:

- (i) TCIN to conduct the MRI scan and store MRI scan data of participant;
- (ii) TCIN or Principal Investigator, (PI) to contact participants GP;
- (iii) TCIN radiographer to send MRI scan data to radiologist acting for TCIN;
- (iv) Radiologist to store data in a hospital system with same care as other patient data ensuring participants confidentiality;
- (v) Radiologist/ Clinician (acting for TCIN) to contact participants GP;
- (vi) TCIN to store data on the study for a period of at least 5 years or as specified in the specific consent form.

A dated standard letter signed by the appropriate Principal Investigator will be sent to all participants GP's, it is the responsibility of the Principal Investigator to ensure that this is sent at least two days before scanning to allow for postal delays. The principal investigator is responsible for their project at all times.

The TCIN designated radiologist will be sent data in a form that allows identification so that if a response is required he can act quickly. This will be stored in the hospital system with the same rigour and attention to confidentiality as all other medical data, as per the rules of that institution. The raw scan data will be stored at TCIN in anonymous form for research purposes as agreed on the consent form of the specific research project.

I agree to the above points and understand that my data will be treated carefully at TCIN and in the hospital system.

Participant Name and Address _____

Signed by Participant _____

APPENDIX 2.3. GP LETTER

Participants GP Name and Address _____

Date: _____

Below (i) is the standard letter to be sent to all participants GP's:



(i) Standard letter to GP from Trinity College Institute of Neuroscience.

Participant ' _____ ' is taking part in a research study, utilising an MRI scanner at Trinity College Institute of Neuroscience, TCD Dublin 2. These research scans, although not full clinical scans, will be read by a clinician.

In the event of an irregularity being found, the clinician, [Dr William Torreggiani of The Adelaide and Meath Hospital Incorporating the National Children's Hospital] will inform you as the participants GP that a proper clinical scan may be required to determine whether or not an irregularity is of clinical significance.

The GP should then contact the participant to advise them that a proper clinical scan, at a hospital, is recommended to check the irregularity.

In the unlikely event that you need to contact us, the following telephone number can be used. (087-347-0032).

This letter was posted to the participants GP on ____/____/____ (Day/Month/Year). Copy of this to be given to TCIN radiographer before scanning takes place.

Signature of PI. _____

APPENDIX 2.4. MRI CONSENT AND CHECKLIST

MAGNETIC RESONANCE IMAGING



Name: _____

Phone- _____

Sex: Male [☐] Female [☐]

Date of Birth: _____

Weight: _____ kg

Please provide us with the details of another person (e.g., next-of-kin) should we need to contact you in the future.

Name of contact person: _____

Phone: _____

Study: New approaches to functional Magnetic Resonance Imaging in the human brain.

Time in: ____:____ Time Out: ____:____

Investigator: _____

CHECK LIST FOR 3T MAGNETIC RESONANCE IMAGING

Do you have any of the following:

Cardiac Pacemaker:	Yes	No
Have you ever had any surgical procedures?	Yes	No

If yes, what type and where

.....

.....

.....

.....

The following items may be harmful to you during your MR scan or may interfere with the MR examination. You must provide a “yes” or “no” for every item. Please indicate if you have or have had any of the following:

Yes	No	
___	___	Any type of electronic, mechanical, or magnetic implant Type:
___	___	Cardiac pacemaker
___	___	Aneurysm clip
___	___	Implanted cardiac defibrillator
___	___	Neurostimulator
___	___	Biostimulator Type:
___	___	Any type of internal electrodes or wires
___	___	Cochlear implant
___	___	Hearing aid
___	___	Implanted drug pump (eg, insulin, Baclofen, chemotherapy, pain medicine)
___	___	Halo vest
___	___	Spinal fixation device
___	___	Spinal fusion procedure
___	___	Any type of coil, filter, or stent Type:
___	___	Any type of metal object (eg, shrapnel, bullet, BB)
___	___	Artificial heart valve
___	___	Any type of ear implant
___	___	Penile implant
___	___	Artificial eye
___	___	Eyelid spring
___	___	Any type of implant held in place by a magnet Type:
___	___	Any type of surgical clip or staple
___	___	Any IV access port (eg, Broviac, Port-a-Cath, Hickman, Picc line)
___	___	Medication patch (eg, nitroglycerine, nicotine)
___	___	Shunt
___	___	Artificial limb or joint What and where:
___	___	Tissue expander (eg, breast)
___	___	Removable dentures, false teeth, or partial plate
___	___	Diaphragm, IUD, Pessary Type:
___	___	Surgical mesh Location:
___	___	Body piercing Location:
___	___	Wig, hair implants
___	___	Tattoos or tattooed eyeliner

Information:

The MRI scan being done is designed to answer research questions, not examine your brain medically. This MRI scan is not a substitute for one a doctor would order. It may not show problems that would be picked up by a medical MRI scan.

There are some items that may interfere with the Magnetic Resonance Imaging and some that may be potentially hazardous. To help us to determine your suitability for an MRI scan and to ensure your safety, please complete the following checklist carefully. Not all people can have an MRI scan because the strong magnetic field may be hazardous to them.

- People with
 - permanent pacemakers
 - prosthetic heart valves
 - implanted cardiac defibrillators
 - certain types of vascular clips
- Cannot have an MRI scan**

Instructions for Patient/Volunteers

1. You are urged to use the earplugs or headphones we supply during your MRI examination since some patients may find the noise levels unacceptable, and the noise levels may affect your hearing.
2. Remove all jewelry (eg, necklaces, pins, rings).
3. Remove all hairpins, bobby pins, barrettes, clips, etc.
4. Remove all dentures, false teeth, and partial dental plates.
5. Remove hearing aides.
6. Remove eyeglasses.
7. Remove your watch, pager, cell phone, credit cards, bankcards, and all other cards with a magnetic strip.
8. Remove body piercing objects.
9. Use gown, if provided, or remove all clothing with metal fasteners, zippers, etc.

_____ Claustrophobia
Native Language: _____

For female participants it is also important that you tell us if there is any possibility that you are pregnant. To date there are no known risks of MRI during pregnancy, however as a precautionary safety measure pregnant individuals will not be included in the study. To participate in the current study women of child-bearing potential must be using one of the following acceptable methods of birth-control:

- a. oral or transdermal contraceptives
- b. barrier (diaphragm or condom) with spermicide
- c. intrauterine progesterone contraceptive system
- d. levonorgestrel implant
- e. medroxyprogesterone acetate contraceptive injection
- f. complete abstinence from sexual activity

Do you meet these requirements?

Yes No

I have read and understood this form, and consent to my study being used for research.

I have had a chance to ask any questions.

Signed (Volunteer): _____

Witnessed (Researcher/MR staff): _____

Date: _____

APPENDIX 3.1. MINI STRUCTURED CLINICAL INTERVIEW FOR DSM

NAME: _____ DATE: _____

A. MAJOR DEPRESSIVE EPISODE

(→ MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE NO IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

A1	Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?	NO	YES
A2	In the past two weeks, have you been much less interested in most things or much less able to enjoy the things you used to enjoy most of the time?	NO	YES
	IS A1 OR A2 CODED YES?	→NO	YES

A3 Over the past two weeks, when you felt depressed or uninterested:

- | | | | |
|---|--|----|-------|
| a | Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e., by $\pm 5\%$ of body weight or ± 8 lbs. or ± 3.5 kgs., for a 160 lb./70 kg. person in a month)?
IF YES TO EITHER, CODE YES. | NO | YES * |
| b | Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)? | NO | YES |
| c | Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still almost every day? | NO | YES * |
| d | Did you feel tired or without energy almost every day? | NO | YES |
| e | Did you feel worthless or guilty almost every day? | NO | YES |
| f | Did you have difficulty concentrating or making decisions almost every day? | NO | YES |
| g | Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead? | NO | YES |

ARE 5 OR MORE ANSWERS (A1-A3) CODED YES?

NO YES *

**MAJOR DEPRESSIVE
EPISODE, CURRENT**

IF PATIENT HAS CURRENT MAJOR DEPRESSIVE EPISODE CONTINUE TO A4,
OTHERWISE MOVE TO MODULE B:

- A4 a During your lifetime, did you have other episodes of two weeks or more when you felt depressed or uninterested in most things, and had most of the problems we just talked about? →NO YES

- b In between 2 episodes of depression, did you ever have an interval of at least 2 months, without any depression and any loss of interest?

NO YES

**MAJOR DEPRESSIVE
EPISODE, RECURRENT**

* If patient has Major Depressive Episode, Current, use this information in coding the corresponding questions on page 5 (A6d, A6e).

MAJOR DEPRESSIVE EPISODE WITH MELANCHOLIC FEATURES (optional)

(➔ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

IF THE PATIENT CODES POSITIVE FOR A CURRENT MAJOR DEPRESSIVE EPISODE (A3 = YES), EXPLORE THE FOLLOWING:

A5	a	During the most severe period of the current depressive episode, did you lose almost completely your ability to enjoy nearly everything?	NO	YES
	b	During the most severe period of the current depressive episode, did you lose your ability to respond to things that previously gave you pleasure, or cheered you up? IF NO: When something good happens does it fail to make you feel better, even temporarily?	NO	YES
		IS EITHER A5a OR A5b CODED YES?	➔NO	YES

A6 Over the past two week period, when you felt depressed and uninterested:

a	Did you feel depressed in a way that is different from the kind of feeling you experience when someone close to you dies?	NO	YES
b	Did you feel regularly worse in the morning, almost every day?	NO	YES
c	Did you wake up at least 2 hours before the usual time of awakening and have difficulty getting back to sleep, almost every day?	NO	YES
d	IS A3c CODED YES (PSYCHOMOTOR RETARDATION OR AGITATION)?	NO	YES
e	IS A3a CODED YES FOR ANOREXIA OR WEIGHT LOSS?	NO	YES
f	Did you feel excessive guilt or guilt out of proportion to the reality of the situation?	NO	YES

ARE 3 OR MORE A6 ANSWERS CODED YES?

NO	YES
Major Depressive Episode with Melancholic Features Current	

B. DYSTHYMIA

(➔ MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

IF PATIENT'S SYMPTOMS CURRENTLY MEET CRITERIA FOR MAJOR DEPRESSIVE EPISODE, DO NOT EXPLORE THIS MODULE.

B1	Have you felt sad, low or depressed most of the time for the last two years?	➔NO	YES
B2	Was this period interrupted by your feeling OK for two months or more?	NO	➔YES
B3	During this period of feeling depressed most of the time:		
a	Did your appetite change significantly?	NO	YES
b	Did you have trouble sleeping or sleep excessively?	NO	YES
c	Did you feel tired or without energy?	NO	YES
d	Did you lose your self-confidence?	NO	YES
e	Did you have trouble concentrating or making decisions?	NO	YES
f	Did you feel hopeless?	NO	YES
	ARE 2 OR MORE B3 ANSWERS CODED YES?	➔NO	YES

B4Did the symptoms of depression cause you significant distress or impair your ability to function at work, socially, or in some other important way?

NO	YES
DYSTHYMIA CURRENT	

C. SUICIDALITY

In the past month did you:

				Points
C1	Suffer any accident?	NO	YES	0
	IF NO TO C1, SKIP TO C2; IF YES, ASK C1a,:			
C1a	Plan or intend to hurt yourself in that accident either passively or actively?	NO	YES	0
	IF NO TO C1a, SKIP TO C2; IF YES, ASK C1b,:			
C1b	Did you intend to die as a result of this accident?	NO	YES	0
C2	Think that you would be better off dead or wish you were dead?	NO	YES	1
C3	Want to harm yourself or to hurt or to injure yourself?	NO	YES	2
C4	Think about suicide?	NO	YES	6

IF YES, ASK ABOUT THE INTENSITY AND FREQUENCY OF THE SUICIDAL IDEATION:

	Frequency	Intensity				
	Occasionally	Mild	→	Can you control these impulses and state that you will not act on them while in this program? Only score 8 points if response is NO.		
	Often	Moderate				
	Very often	Severe				
				NO	YES	8
C5	Have a suicide plan?			NO	YES	8
C6	Take any active steps to prepare to injure yourself or to prepare for a suicide attempt in which you expected or intended to die?			NO	YES	9
C7	Deliberately injure yourself without intending to kill yourself?			NO	YES	4
C8	Attempt suicide?			NO	YES	10
	Hoped to be rescued / survive					
	Expected / intended to die					

In your lifetime:

C9	Did you ever make a suicide attempt?	NO	YES	4
----	--------------------------------------	----	-----	---

IS AT LEAST 1 OF THE ABOVE (EXCEPT C1) CODED YES?

IF YES, ADD THE TOTAL NUMBER OF POINTS FOR THE ANSWERS (C1-C9)
CHECKED 'YES' AND SPECIFY THE LEVEL OF SUICIDE RISK AS
INDICATED IN THE DIAGNOSTIC BOX:

MAKE ANY ADDITIONAL COMMENTS ABOUT YOUR ASSESSMENT
OF THIS PATIENT'S CURRENT AND NEAR FUTURE SUICIDE RISK IN
THE SPACE BELOW:

NO **YES**

SUICIDE RISK CURRENT

1-8 points Low
9-16 points Moderate
≥ 17 points High

APPENDIX 3.2. HAMILTON DEPRESSION RATING SCALE

Patient Name: _____

Date: _____

Hamilton Rating Scale for Depression (17-items)

Instructions: For each item select the "cue" which best characterizes the patient during the past week.

1. **Depressed Mood**
(sadness, hopeless, helpless, worthless)
 - 0 Absent
 - 1 These feeling states indicated only on questioning
 - 2 These feeling states spontaneously reported verbally
 - 3 Communicates feeling states nonverbally, i.e., through facial expression, posture, voice and tendency to weep
 - 4 Patient reports VIRTUALLY ONLY these feeling states in his spontaneous verbal and nonverbal communication
2. **Feelings of Guilt**
 - 0 Absent
 - 1 Self-reproach, feels he has let people down
 - 2 Ideas of guilt or rumination over past errors or sinful deeds
 - 3 Present illness is a punishment. Delusions of guilt
 - 4 Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations
3. **Suicide**
 - 0 Absent
 - 1 Feels life is not worth living
 - 2 Wishes he were dead or any thoughts of possible death to self
 - 3 Suicide ideas or gesture
 - 4 Attempts at suicide (any serious attempt rates 4)
4. **Insomnia - Early**
 - 0 No difficulty falling asleep
 - 1 Complains of occasional difficulty falling asleep i.e., more than ½ hour
 - 2 Complains of nightly difficulty falling asleep
5. **Insomnia - Middle**
 - 0 No difficulty
 - 1 Patient complains of being restless and disturbed during the night
 - 2 Waking during the night – any getting out of bed rates 2 (except for purposes of voiding)
6. **Insomnia - Late**
 - 0 No difficulty
 - 1 Waking in early hours of the morning but goes back to sleep
 - 2 Unable to fall asleep again if gets out of bed
7. **Work and Activities**
 - 0 No difficulty
 - 1 Thoughts and feelings of incapacity, fatigue or weakness related to activities; work or hobbies
 - 2 Loss of interest in activity; hobbies or work – either directly reported by patient, or indirect in listlessness, indecision and vacillation (feels he has to push self to work or activities)
 - 3 Decrease in actual time spent in activities or decrease in productivity. In hospital, rate 3 if patient does not spend at least three hours a day in activities (hospital job or hobbies) exclusive of ward chores.
 - 4 Stopped working because of present illness. In hospital, rate 4 if patient engages in no activities except ward chores, or if patient fails to perform ward chores unassisted.
8. **Retardation**
(slowness of thought and speech; impaired ability to concentrate; decreased motor activity)
 - 0 Normal speech and thought
 - 1 Slight retardation at interview
 - 2 Obvious retardation at interview
 - 3 Interview difficult
 - 4 Complete stupor
9. **Agitation**
 - 0 None
 - 1 "Playing with" hand, hair, etc.
 - 2 Hand-wringing, nail-biting, biting of lips
10. **Anxiety - Psychic**
 - 0 No difficulty
 - 1 Subjective tension and irritability
 - 2 Worrying about minor matters
 - 3 Apprehensive attitude apparent in face or speech
 - 4 Fears expressed without questioning
11. **Anxiety - Somatic**
 - 0 Absent Physiological concomitants of anxiety such as:
 - 1 Mild Gastrointestinal - dry mouth, wind, indigestion,
 - 2 Moderate diarrhea, cramps, belching
 - 3 Severe Cardiovascular – palpitations, headaches
 - 4 Incapacitating Respiratory - hyperventilation, sighing
 - Urinary frequency
 - Sweating
12. **Somatic Symptoms - Gastrointestinal**
 - 0 None
 - 1 Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen.
 - 2 Difficulty eating without staff urging. Requests or requires laxatives or medications for bowels or medication for G.I. symptoms.
13. **Somatic Symptoms - General**
 - 0 None
 - 1 Heaviness in limbs, back or head, backaches, headache, muscle aches, loss of energy and fatigability
 - 2 Any clear-cut symptom rates 2
14. **Genital Symptoms**
 - 0 Absent 0 Not ascertained
 - 1 Mild Symptoms such as: loss of libido,
 - 2 Severe menstrual disturbances
15. **Hypochondriasis**
 - 0 Not present
 - 1 Self-absorption (bodily)
 - 2 Preoccupation with health
 - 3 Frequent complaints, requests for help, etc.
 - 4 Hypochondriacal delusions
16. **Loss of Weight**
 - A. When Rating by History:
 - 0 No weight loss
 - 1 Probable weight loss associated with present illness
 - 2 Definite (according to patient) weight loss
 - B. On Weekly Ratings by Ward Psychiatrist, When Actual Changes are Measured:
 - 0 Less than 1 lb. weight loss in week
 - 1 Greater than 1 lb. weight loss in week
 - 2 Greater than 2 lb. weight loss in week
17. **Insight**
 - 0 Acknowledges being depressed and ill
 - 1 Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
 - 2 Denies being ill at all

Total Score: _____

APPENDIX 3.3. HAMILTON ANXIETY RATING SCALE

Hamilton Anxiety Rating Scale (HAM-A)

Below is a list of phrases that describe certain feeling that people have. Rate the patients by finding the answer which best describes the extent to which he/she has these conditions. Select one of the five responses for each of the fourteen questions.

0 = Not present, 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Very severe.

1 Anxious mood ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Worries, anticipation of the worst, fearful anticipation, irritability.

2 Tension ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Feelings of tension, fatigability, startle response, moved to tears easily, trembling, feelings of restlessness, inability to relax.

3 Fears ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Of dark, of strangers, of being left alone, of animals, of traffic, of crowds.

4 Insomnia ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Difficulty in falling asleep, broken sleep, unsatisfying sleep and fatigue on waking, dreams, nightmares, night terrors.

5 Intellectual ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Difficulty in concentration, poor memory.

6 Depressed mood ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Loss of interest, lack of pleasure in hobbies, depression, early waking, diurnal swing.

7 Somatic (muscular) ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Pains and aches, twitching, stiffness, myoclonic jerks, grinding of teeth, unsteady voice, increased muscular tone.

8 Somatic (sensory) ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Tinnitus, blurring of vision, hot and cold flushes, feelings of weakness, pricking sensation.

9 Cardiovascular symptoms ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Tachycardia, palpitations, pain in chest, throbbing of vessels, fainting feelings, missing beat.

10 Respiratory symptoms ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Pressure or constriction in chest, choking feelings, sighing, dyspnea.

11 Gastrointestinal symptoms ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Difficulty in swallowing, wind abdominal pain, burning sensations, abdominal fullness, nausea, vomiting, borborygmi, looseness of bowels, loss of weight, constipation.

12 Genitourinary symptoms ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Frequency of micturition, urgency of micturition, amenorrhea, menorrhagia, development of frigidity, premature ejaculation, loss of libido, impotence.

13 Autonomic symptoms ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Dry mouth, flushing, pallor, tendency to sweat, giddiness, tension headache, raising of hair.

14 Behavior at interview ☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4

Fidgeting, restlessness or pacing, tremor of hands, furrowed brow, strained face, sighing or rapid respiration, facial pallor, swallowing, etc.

APPENDIX 3.4. RUMINATION REFLECTION QUESTIONNAIRE

RRQ

Instructions:

For each of the statements located on the next two pages, please indicate your level of agreement or disagreement by circling one of the scale categories to the right of each statement. Use the scale as shown below:

	Strongly Disagree 1	Disagree 2	Neutral 3	Agree 4	Strongly Agree 5
1. My attention is often focused on aspects of myself I wish I'd stop thinking about.....	1	2	3	4	5
2. I always seem to be "re-hashing" in my mind recent things I've said or done.....	1	2	3	4	5
3. Sometimes it is hard for me to shut off thoughts about myself.	1	2	3	4	5
4. Long after an argument or disagreement is over with, my thoughts keep going back to what happened.	1	2	3	4	5
5. I tend to "ruminate" or dwell over things that happen to me for a really long time afterward.	1	2	3	4	5
6. I don't waste time re-thinking things that are over and done with.	1	2	3	4	5
7. Often I'm playing back over in my mind how I acted in a past situation.	1	2	3	4	5
8. I often find myself re-evaluating something I've done.	1	2	3	4	5
9. I never ruminate or dwell on myself for very long.	1	2	3	4	5
10. It is easy for me to put unwanted thoughts out of my mind.	1	2	3	4	5
11. I often reflect on episodes in my life that I should no longer concern myself with.	1	2	3	4	5
12. I spend a great deal of time thinking back over my embarrassing or disappointing moments.	1	2	3	4	5
13. Philosophical or abstract thinking doesn't appeal to me that much.....	1	2	3	4	5
14. I'm not really a meditative type of person.	1	2	3	4	5
15. I love exploring my "inner" self.	1	2	3	4	5
16. My attitudes and feelings about things fascinate me.	1	2	3	4	5
17. I don't really care for introspective or self-reflective thinking.	1	2	3	4	5
18. I love analyzing why I do things.	1	2	3	4	5
19. People often say I'm a "deep", introspective type of person.	1	2	3	4	5
20. I don't care much for self-analysis.	1	2	3	4	5
21. I'm very self-inquisitive by nature.	1	2	3	4	5
22. I love to meditate on the nature and meaning of things.	1	2	3	4	5
23. I often love to look at my life in philosophical ways.....	1	2	3	4	5
24. Contemplating myself isn't my idea of fun.	1	2	3	4	5

APPENDIX 3.5. PITTSBURGH SLEEP QUALITY INDEX

Pittsburgh Sleep Quality Index (PSQI)

Name: _____

Age: _____

Gender: _____ Male _____ Female

Date: _____

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. When have you usually gotten up in the morning? _____
4. How many hours of actual sleep do you get at night? (This may be different than the number of hours you spend in bed) _____

5. During the past month, how often have you had trouble sleeping because you...	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason:				
6. During the past month, how often have you taken medicine (prescribed or "over the counter" to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)
9. During the past month, how would you rate your sleep quality overall?				

APPENDIX 3.5. CHILDHOOD TRAUMA QUESTIONNAIRE

CTQ

Name: _____

Age: _____

Gender: _____ Male _____ Female

When I was growing up...	Never True	Rarely True	Sometimes True	Often True	Very Often True
1. I didn't have enough to eat.					
2. I knew that there was someone to take care of me and protect me					
3. People in my family called me things like "stupid," "lazy," or "ugly."					
4. My parents were too drunk or high to take care of the family.					
5. There was someone in my family who helped me feel that I was important or special.					
6. I had to wear dirty clothes.					
7. I felt loved.					
8. I thought that my parents wished I had never been born.					
9. I got hit so hard by someone in my family that I had to see a doctor or go to the hospital.					
10. There was nothing I wanted to change about my family.					
11. People in my family hit me so hard that it left me with bruises or marks.					
12. I was punished with a belt, a board, a cord, or some other hard object.					
13. People in my family looked out for each other.					
14. People in my family said hurtful or insulting things to me.					
15. I believe that I was physically abused.					
16. I had the perfect childhood.					
17. I got hit or beaten so badly that it was noticed by someone like a teacher, neighbor, or doctor.					
18. I felt that someone in my family hated me.					
19. People in my family felt close to each other.					
20. Someone tried to touch me in a sexual way, or tried to make me touch them.					
21. Someone threatened to hurt me or tell lies about me unless I did something sexual with them.					
22. I had the best family in the world.					
23. Someone tried to make me do sexual things or watch sexual things.					
24. Someone molested me.					
25. I believe that I was emotionally abused.					
26. There was someone to take me to the doctor if I needed it.					
27. I believe that I was sexually abused.					
28. My family was a source of strength and support.					

APPENDIX 4. EXTRA TABLES

Hippocampal T1-only data.

The new FreeSurfer version uses both T1 and T2-FLAIR data to demarcate hippocampal substructures. The previous version, 5.3, relied on T1 data only. I also processed the T1 only data through the pipeline as a comparison of the old and new version. Comparisons between total depressed and controls only.

	Left			Right		
	Con vs Dep	Con vs FPD	Con vs RD	Con vs Dep	Con vs FPD	Con vs RD
Substructure	<i>P</i> -value			<i>P</i> -value		
CA1	0.007	0.036	0.074	0.604	0.511	0.949
CA2/3	0.0003	0.002	0.013	0.027	0.033	0.226
CA4	0.006	0.076	0.009	0.025	0.085	0.083
Dentate	0.031	0.307	0.009	0.046	0.101	0.158
Subiculum	0.189	0.7	0.041	0.609	0.653	0.208
Tail	0.154	0.459	0.081	0.772	0.595	0.975
Molecular Layer	0.003	0.056	0.006	0.158	0.369	0.242
Presubiculum	0.5365	0.426	0.984	0.392	0.123	0.941
Parasubiculum	0.734	0.88	0.794	0.273	0.777	0.147
Fimbria	0.371	0.387	0.644	0.701	0.543	0.984
Fissure	0.419	0.995	0.179	0.081	0.743	0.007
HATA	0.168	0.286	0.289	0.824	0.663	0.601
Composite Measures						
Hippocampal Extended	0.006	0.107	0.005	0.321	0.465	0.475
Hippocampal Formation	0.002	0.055	0.002	0.172	0.381	0.267
Hippocampal Proper	0.002	0.02	0.023	0.114	0.182	0.374
D-Complex	0.003	0.061	0.004	0.035	0.112	0.101
C-Complex	0.003	0.052	0.06	0.283	0.303	0.689
M-Complex	0.003	0.072	0.003	0.267	0.552	0.326

Inter-Rater Reliability for fornix-stria protocol

The ICC between each rater for dimension and diffusion measurements of the fornix-stria demonstrated excellent inter-rater reliability, with a value of 0.93 over all the measurements. For tract diffusion metrics, the ICCs exceeded 0.97 in all tracts. Inter-rater ICCs are presented below.

	Rater Mean	1 Rater Mean	2 ICC	95% CI Lower Bound	Upper Bound
Dimension s					
Number of Tracts	855.014	814.286	0.933	0.895	0.958
Length (mm)	65.935	65.207	0.882	0.817	0.925
Volume (mm ³)	7824.038	7120.581	0.797	0.692	0.869
Diffusion Metrics					
FA	0.4495	0.4489	0.978	0.964	0.986
MD (mm ² /2)	0.00115	0.00116	0.984	0.975	0.99
AD (mm ² /2)	0.00139	0.00139	0.984	0.974	0.99
RD (mm ² /2)	0.001008	0.001009	0.983	0.972	0.989

Each rater was compared for dimension and diffusion measurements of the fornix-stria combination. The upper and lower limits of data within the 95% confidence interval are also shown. Statistical significance between groups is indicated by $p < 0.05$. AD, Axial Diffusivity; CI, Confidence Interval; FA, Fractional Anisotropy; ICC, Inter-rater correlation coefficient; MD, Mean Diffusivity.