University of Alberta

Hippocampal neuroplasticity and neurogenesis in major depressive disorder: a high field MRI study

by

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Dedication

To my parents, who have made many sacrifices for me to be where I am today, your care throughout a lifetime's journey across the globe, constant support for my endeavours and faith in my decisions even from over a thousand miles away has fueled my motivation to push myself to persevere and continue aiming higher.

And to my friends and colleagues, both locally in the university and afar, who have been another source of constant moral support for continuously encouraging me and not giving up hope in me.

Abstract

The hippocampus is a brain structure responsible for memory, learning, and the stress response; it is also used as a model for major depressive disorder (MDD) in preclinical studies. Preclinical models have shown that the hippocampal subfields are differentially affected by chronic stress. Studies using magnetic resonance imaging (MRI) have shown reductions in hippocampal volumes MDD.

With the high-field 4.7 Tesla MRI, we have for the first time analyzed the hippocampal subfields *in vivo* in patients with MDD and healthy controls. Our data suggest that MDD patients had smaller volumes of the cornu ammonis (CA) and dentate gyrus (DG) subfields of the hippocampus, which contributed to an overall reduction in the total volume of the hippocampus and its subregions. Our results also suggested that antidepressant treatment might reverse these volumetric reductions in the CA and DG subfields as suggested previously in preclinical studies.

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List of Abbreviations

2-D	Two-Dimensional
3-D	Three-Dimensional
5-HT	5 - Hydroxytryptamine (also: serotonin)
5-HT1A	5 - Hydroxytryptamine Receptor, Subtype 1A
5-HT2A	5 - Hydroxytryptamine Receptor, Subtype 2A
5-HTT	5 - Hydroxytryptamine Transporter
ACTH	Adrenocorticotropic Hormone (also: corticotrophin)
ANCOVA	Analysis of Covariance
BDNF	Brain-Derived Neurotrophic Factor
BOLD	Blood Oxygen Level Dependent
BrdU	Bromodeoxyuridine
BZ	Benzodiazepine
CA (1-4)	Cornu Ammonis (Areas 1-4)
CIS	Chronic Immobilization Stress
cm	Centimetre(s)
CREB	Cyclic Adenosine Monophosphate Response Element Binding
CRH	Corticotrophin-Releasing Hormone
CT (CAT)	Computed (Axial) Tomography
CTQ	Childhood Trauma Questionnaire
CSF	Cerebrospinal Fluid
CV	Coefficient of Variation
d.f.	Degrees of Freedom

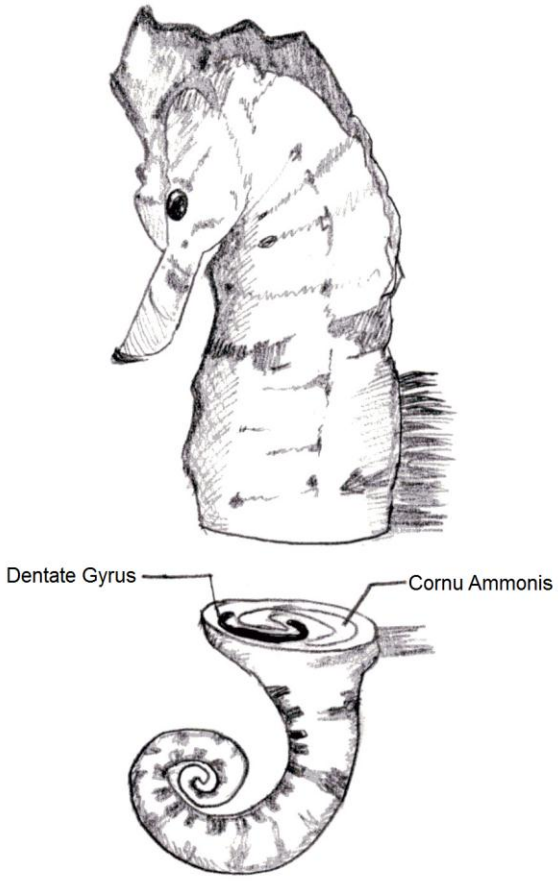
DG	Dentate Gyrus
Diag.	Diagnosis
DSM (IV)	Diagnostic and Statistical Manual of Mental Disorders (fourth edition)
DTM	Diffusion Tensor Microscopy
ECS	Electroconvulsive Shock
ECT	Electroconvulsive Therapy
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FSE	Fast Spin Echo (sequence)
GABA	γ-Aminobutyric Acid
GR	Glucocorticoid Receptor
GRE	Gradient-Recalled Echo (sequence)
HC	Hippocampus
HDRS	Hamilton Depression Rating Scale
HIOC	N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide
HPA	Hypothalamic-Pituitary-Adrenal (axis)
ICC	Intraclass Correlation Coefficient
ICV	Intracranial Volume
MAO	Monoamine Oxidase
MAOI	Monoamine Oxidase Inhibitor
MASQ	Mood and Anxiety Symptom Questionnaire
MCI	Mild Cognitive Impairment
MDD	Major Depressive Disorder

MDE	Major Depressive Episode
mm	Millimetre(s)
MNI	Montreal Neurological Institute
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo (sequence)
MR	Mineralocorticoid Receptor
MRI	Magnetic Resonance Imaging
MRM	Magnetic Resonance Microscopy
ms	Millisecond(s)
MTL	Medial Temporal Lobe
NAS	N-Acetylserotonin
NERI	Norepinephrine Reuptake Inhibitor
NMDA	N-Methyl-D-aspartic acid
NPC	Neural Progenitor Cell
PASW	Predictive Analytics Software
PET	Positron Emission Tomography
PTSD	Post-Traumatic Stress Disorder
SAR	Specific Absorption Rate
SD	Standard Deviation
SE	Spin Echo
SGZ	Subgranular Zone
SNR	Signal-to-Noise Ratio
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SPECT	Single Photon Emission Computed Tomography

SPSS	Statistical Package for the Social Sciences
SSRE	Selective Serotonin Reuptake Enhancer
SSRI	Selective Serotonin Reuptake Inhibitor
Sub	Subiculum
SVZ	Subventricular Zone
T	Tesla
T_1	Longitudinal Relaxation Time
T_2	Transverse Relaxation Time
TCA	Tricyclic Antidepressant
TE	Echo Time
TI	Inversion Recovery Time
TLE	Temporal Lobe Epilepsy
TR	Repetition Time
tx.	Treatment
VBM	Voxel-Based Morphometry
WMS (IV)	Wechsler Memory Scale (Fourth Edition)

Chapter 1

Hippocampal Anatomy



1.1 Introduction to the Hippocampus

The hippocampus (HC) is a structure in the medial temporal lobe (MTL), located at the inferior-medial terminal of the cerebral cortex in humans and other primates. Although research on the cortex proper and the HC have a sociological divide in the scientific community with little overlap, the HC is still very anatomically similar to the cortex proper, as it is a continuation of its grey matter (Braitenberg & Schuz, 1991). The brain has two hippocampi, mirrored in each hemisphere. The name for the HC comes from its curved shape, which is similar to that of a seahorse.

Across its transverse plane, the HC is composed of various subfields named the cornu ammonis (CA), dentate gyrus (DG), and subiculum (Sub) (see Figure 1.1), extending laterally from the Sub to the CA while curving back medially from the CA to the DG, similar to a ram's horn. The CA, also considered the "HC proper", is composed of pyramidal neurons which differ from area to area, and thus is divided into four different areas, CA1-4. The DG, or fascia dentata, located in the centre of the human HC, is composed of granular neurons. Finally, the Sub, contiguous with the CA1 and also composed of pyramidal neurons, is an anatomical transition between the HC and the entorhinal cortex (Duvernoy, 2005).

Among different mammalian model organisms, the hippocampal subfields are oriented in different positions. In the rat HC, the CA1 is superior (dorsal) to the CA3, whereas in humans the inverse is true. This is due to differences in the formation of the structure during development (see Figure A.1 in Appendix A).

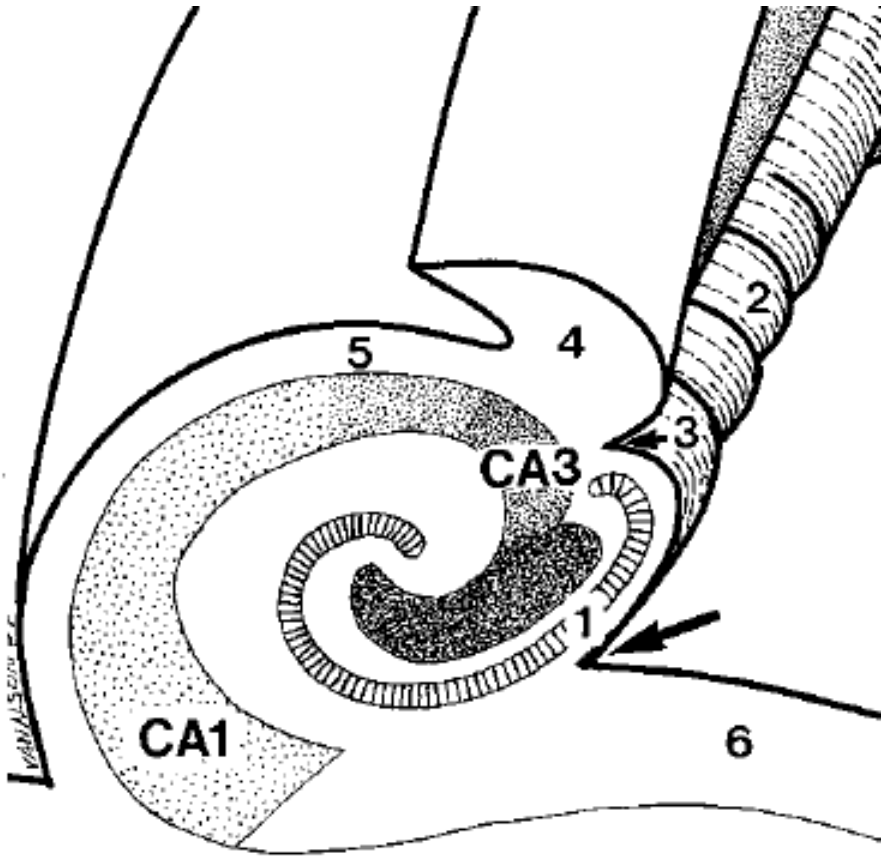


Figure 1.1: Subfields of the HC and surrounding structures

1: DG (Latin: "toothed" ridge), 2: Last dentes of margo denticulatus (Latin: last "tooth" of the DG), 3: Fimbriodentate sulcus (the sulcus between fimbria and the DG), 4: Fimbria (a white matter tract extending across the HC), 5: Alveus ("hollow cavity"), 6: Sub

CA1: CA region 1 (Latin: "Amun's (ram-like) horn"), CA3: CA region 3 (Duvernoy, 2005) (Appendix B)

1.2 Hippocampal Divisions

1.2.1 Longitudinal Divisions (Hippocampal Subregions)

Across the hippocampal longitudinal axis, the HC can be divided into three subregions: the head, body and tail. All three of these subregions contain all three of the subfields which will be discussed below: the CA1-4, DG, and Sub (see Figure 1.2). In humans, the transversely-oriented HC tail (curving medially from the body), is the most superior and posterior part of the HC. The HC body, oriented sagittally, connects the HC tail and the anterior and slightly inferior HC head. The HC head, oriented transversely, curves medially from the HC body while widening in diameter and showing digitations in the boundaries between the subfields .

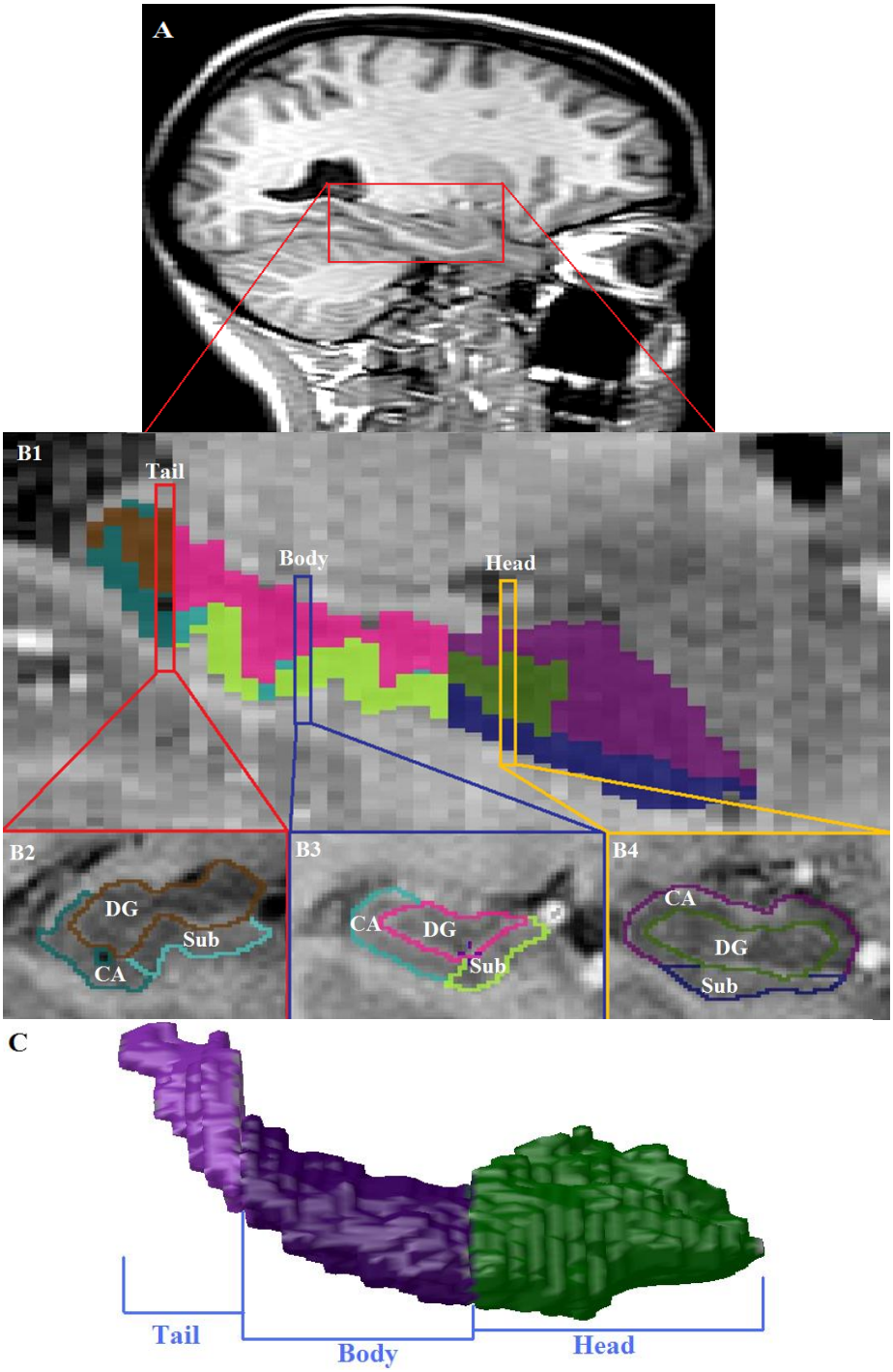


Figure 1.2: A sagittal view of the HC (A) with its location in the brain, (B1) divided into its longitudinal divisions, or hippocampal subregions, along with coronal cross-sections of each subregion: the hippocampal (B2) tail, (B3) body, (B4) head. A 3-D reconstruction taking into account voxels from each slice is also shown in (C).

1.2.2 The Cornu Ammonis

The CA, considered by many neuroanatomists as the "HC proper", envelopes the DG. It is divided into three layers: the cellular elements align in a single, narrow, and dense layer of pyramidal neurons, which are seen after Nissl staining as one dark band flanked on its two sides by lighter bands. On the contrary, the cortex proper has the aforementioned elements scattered throughout the layers into two fainter lines, even though it has very similar neurons – also named pyramidal neurons (Braitenberg & Schuz, 1991) (see Figure 1.3). The three layers of the CA together could be subdivided into six layers: the alveus and stratum oriens as the first light band following Nissl staining, the stratum pyramidale (dark after Nissl staining), and the strata radiatum, lacunosum and moleculare as the other light band (Duvernoy, 2005) (see Figure A.2 top in Appendix A). The pyramidal cells have their soma, which are triangular in the CA1 (with the triangles' bases facing the intraventricular side and apices facing the DG) and ovoid in the CA2, CA3 and CA4, located in the stratum pyramidale. Further toward the ventricular or exterior side of the HC, two more layers lie atop the stratum pyramidale: the stratum oriens and the alveus. In the stratum oriens and pyramidale, soma of basket cells lay scattered while sending arborizations to pyramidal soma, providing inhibitory γ-aminobutyric acid (GABA) inputs to modulate the pyramidal neurons (Olbrich & Braak, 1985). An apical dendrite extends from the pointed end, or apex, of each pyramidal neuron, until it reaches the hippocampal sulcus (a vestigial structure between the CA and DG formed during development and often disappears thereafter) at the end of the stratum

moleculare on the CA side. These apical dendrites, lined parallel to each other, give the stratum radiatum a striated texture. Septal and commissural fibres run along the strata radiatum and lacunosum, the latter stratum also containing perforant fibres from the entorhinal cortex. The strata radiatum and lacunosum, collectively known as the strata lacunosum-moleculare, mark the boundary between the DG and the CA / Sub; they do not technically belong to any single hippocampal subfield as they consist of layers of white matter tracts as opposed to the grey matter of the subfields. The stratum moleculare, also known as the molecular zone, does not contain many neurons aside from the ends of the apical dendrites of the pyramidal neurons and a few interneurons (Duvernoy, 2005). On the other side of the pyramidal soma, many dendrites arborize in the stratum oriens. The neural axon from the soma's basal side crosses the stratum oriens to the alveus, eventually projecting to the septal nuclei in the rostral corpus callosum. These axons also have Schaffer collaterals, which curve back to the other layers to connect with other pyramidal neurons' apical dendrites via the stratum radiatum (Duvernoy, 2005).

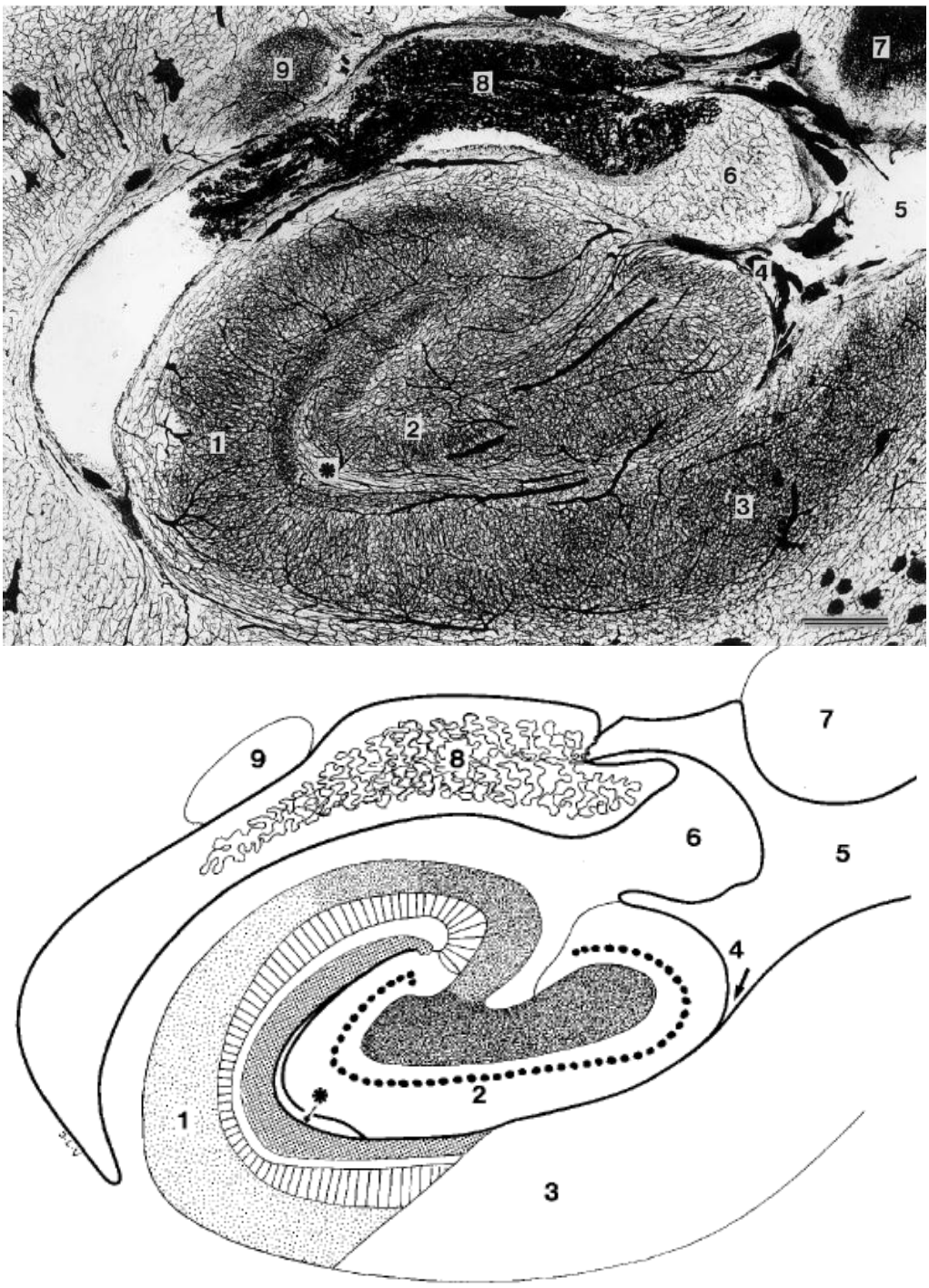


Figure 1.3: The HC and its subfields

Top: Histological staining (intravascular India staining)

Bottom: Sketch of the boundaries (hippocampal body)

1. CA, 2. DG, 3. Sub, 4. Margo denticulatus, 5. Transverse fissure (lateral part), 6. Fimbria, 7. Lateral geniculate body, 8. Lateral ventricle (temporal horn) with choroid plexuses, 9. Caudate nucleus

(Duvernoy, 2005) (Appendix B)

Although pyramidal neurons exist throughout the regions of the CA (CA1, CA2, CA3, and CA4, there are inter-regional differences in cell morphology and organization (see Figure 1.4) (Duvernoy, 2005). The pyramidal neurons of the CA1 tend to be small and scattered with triangular cells (Dam, 1979), while their counterparts in the CA2 and CA3 tend to have rounder, large, ovoid, and densely packed somata (relatively more dense in the CA2). The neurons in the CA1 are most affected in hypoxia, in comparison with the more hypoxia-resistant CA3 cells (Spielmeyer, 1927). The CA3's pyramidal somata, in the curve or genu of the CA, are surrounded by fine, unmyelinated mossy fibres compressed in the stratum lucidum, which is between the strata radiatum and pyramidale (Duvernoy, 2005). A smaller number of CA4 somata are also large and ovoid, scattered among large and myelinated fibres. Since the CA4 is enclosed by the DG, anatomists either include the former as part of the latter by many anatomists, or include both the CA4 and DG as part of the "area dentata" (Blackstad, 1956).

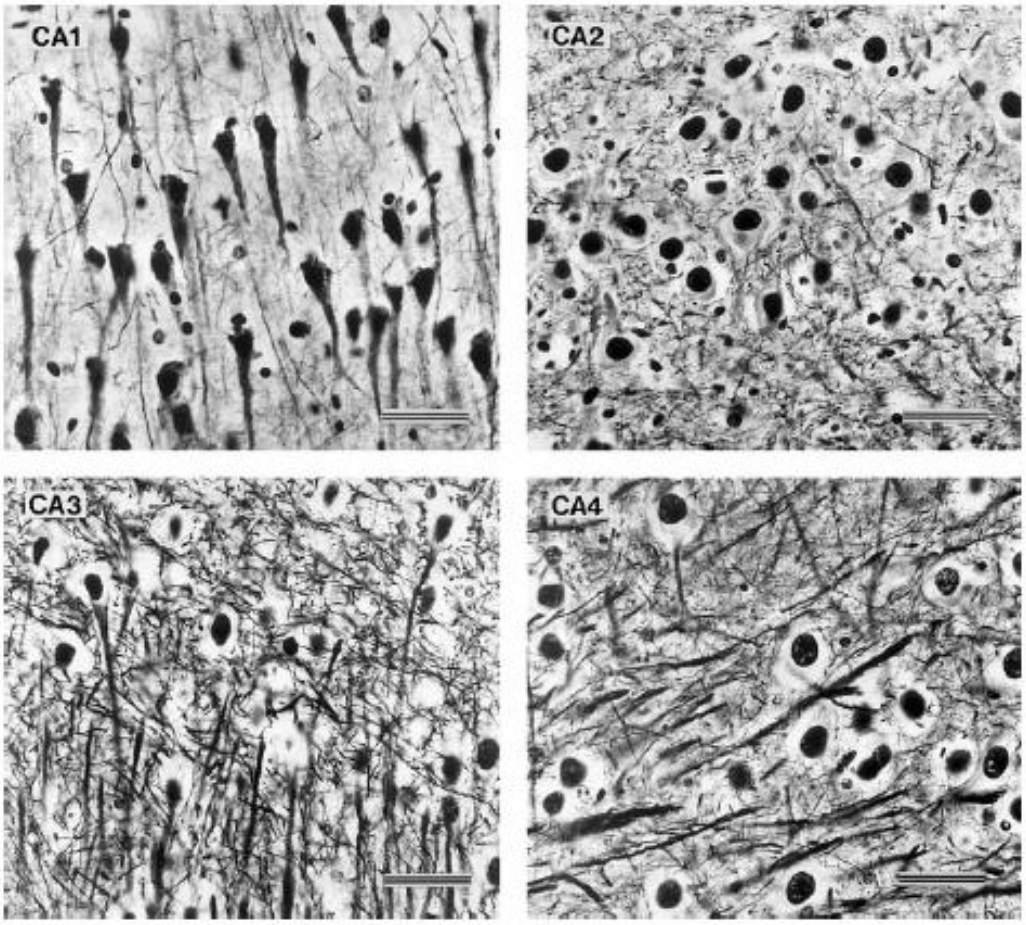


Figure 1.4: Cellular differences between neurons in the regions of the CA.
Top-left – CA1
Top-right – CA2
Bottom-left – CA3
Bottom-right – CA4
(Duvernoy, 2005) (Appendix B)

1.2.3 The Dentate Gyrus

The DG, across the vestigial hippocampal sulcus from the CA, consists of granular neurons with somata in the prominent stratum granulosum, the main layer of this subfield (see Figure A.2 bottom in Appendix A); the prominence of this layer is due to these neurons' densely packed, small, and round somata (see Figure 1.5). The granular neurons receive inputs from neurons from the perforant pathway, coming from the entorhinal cortex, and from commissural and septal fibres, coming from other granular neurons in the stratum granulosum. These inputs all come from the DG's own stratum moleculare, separated from the CA's thinner stratum moleculare by the vestigial hippocampal sulcus. The fibres from the perforant pathway run along the region of the stratum moleculare closer to the hippocampal sulcus (more apically along the granular axon), whereas the fibres from the commissural and septal fibres cross closer to the stratum granulosum (closer to the granular soma) (Cerbone et al., 1993). These fibres connect to a granular neuron via the latter's apical dendrite. On the opposite side of the DG, axons of the granular neurons extend as mossy fibres through the polymorphic, or plexiform, layer onto the CA4 and CA3 (Duvernoy, 2005).

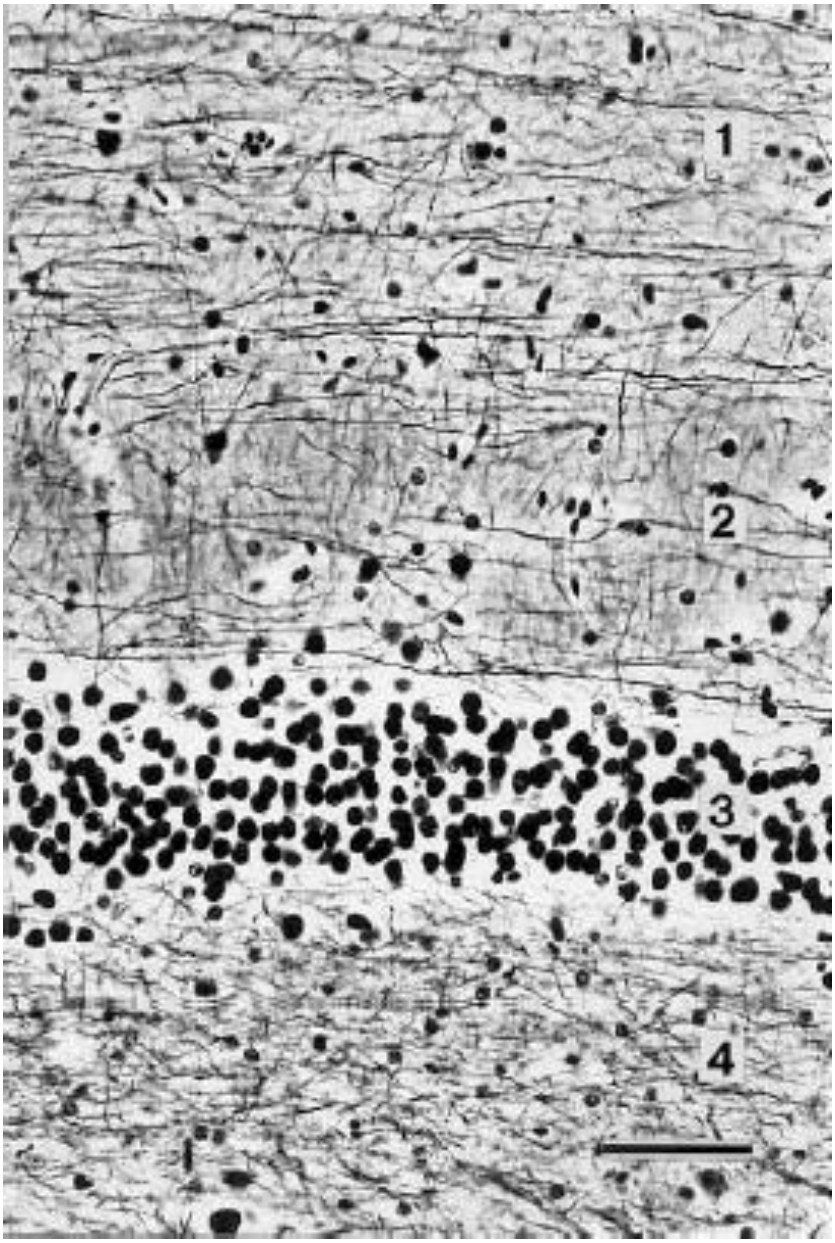


Figure 1.5: The layers of the DG

1. The external / apical third of the stratum moleculare
2. The internal / basal third of the stratum moleculare
3. The stratum granulosum
4. The polymorphic layer

(Duvernoy, 2005) (Appendix B)

1.2.4 The Subiculum

Medially from the CA1, the HC is joined to the Sub, which forms part of the parahippocampal gyrus. The Sub, which extends medially, is divided into the Sub proper, presubiculum, and parasubiculum. The presubiculum is packed with spotted clusters of small pyramidal neurons on its surface (Amaral et al., 1984; Braak & Braak, 1983). The Sub acts as a transitional layer between the six-layered neocortex and the three-layered HC in the CA3 (Kiernan & Barr, 2005). Anatomically, it contains features of both areas. Similar to the cortex proper, the Sub's pyramidal neurons are arranged more diffusely than the CA's, but similar to the CA, the Sub has one layer of pyramidal neurons rather than two, making three total layers rather than five in the entorhinal cortex starting from the presubiculum (see Figure 1.3) (Braitenberg & Schuz, 1991).

Overall, the CA has the largest average area within the HC, while the DG has the second largest. Within the hippocampal tail, the CA's volume tends to predominate, while the volume of the Sub is lowest among the subfields. In the hippocampal body, the volume of the DG is highest, while the volume of the Sub is lowest again. Finally, in the hippocampal head, the CA is larger than the similarly sized DG and Sub (Malykhin et al., 2010a).

1.2.5 Adjacent Structures

Past the Sub, the entorhinal cortex continues along the cortex bilaterally, extending in the inferior direction. The two amygdalas, also functionally related to the HC as part of the limbic system, include grey matter that lies just anterior and superior to the hippocampal head of both hippocampi. Amygdalar connections are involved in olfactory and limbic functions (Aggleton, 1992; Duvernoy, 2005) (see Figures 1.6 and 1.7).

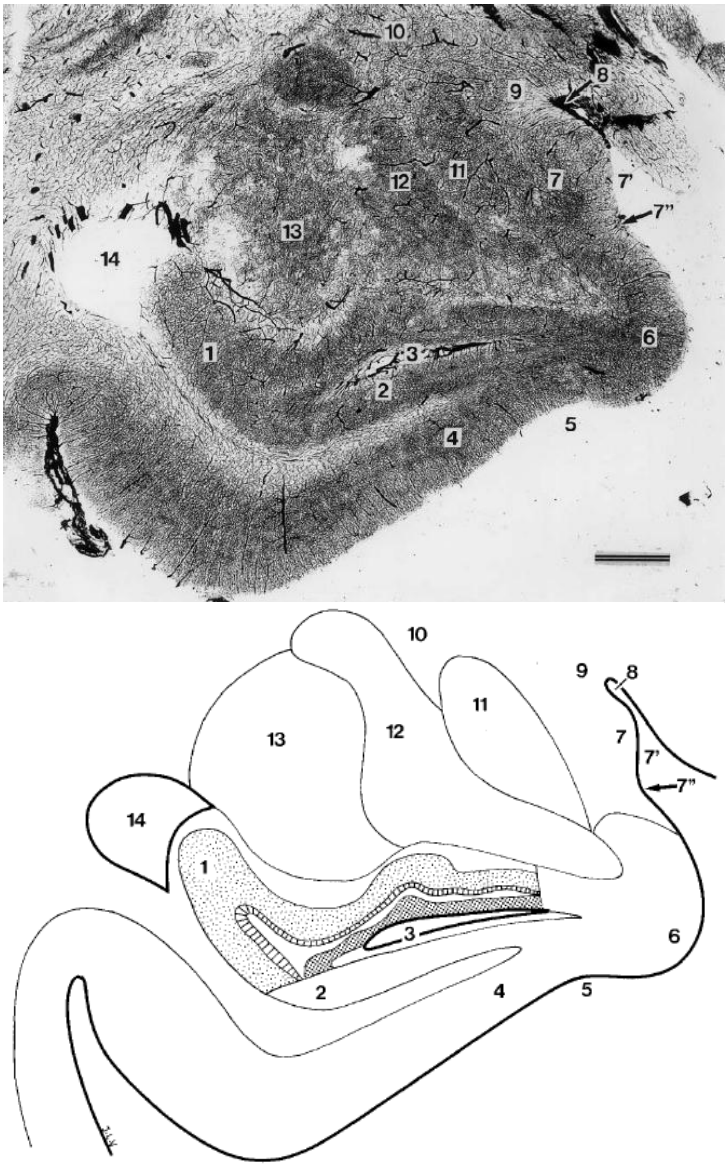


Figure 1.6: The HC and its surrounding structures

Top: Histological staining (intravascular India staining)

Bottom: Sketch of the boundaries (hippocampal head) with the various subfields.

1. CA, 2. Sub, 3. Uncal Sulcus, 4. Parahippocampal gyrus, 5. Uncal notch, 6. Ambient gyrus and entorhinal area, 7-13. Amygdala (7. Cortical nucleus, 7'. Semilunar gyrus, 7" Semianular sulcus, 8. Entorhinal sulcus, 9. Medial nucleus, 10. Central nucleus, 11. Accessory basal nucleus, 12. Basal nucleus, 13. Lateral nucleus), 14. Lateral ventricle (temporal horn)

(Duvernoy, 2005) (Appendix B)

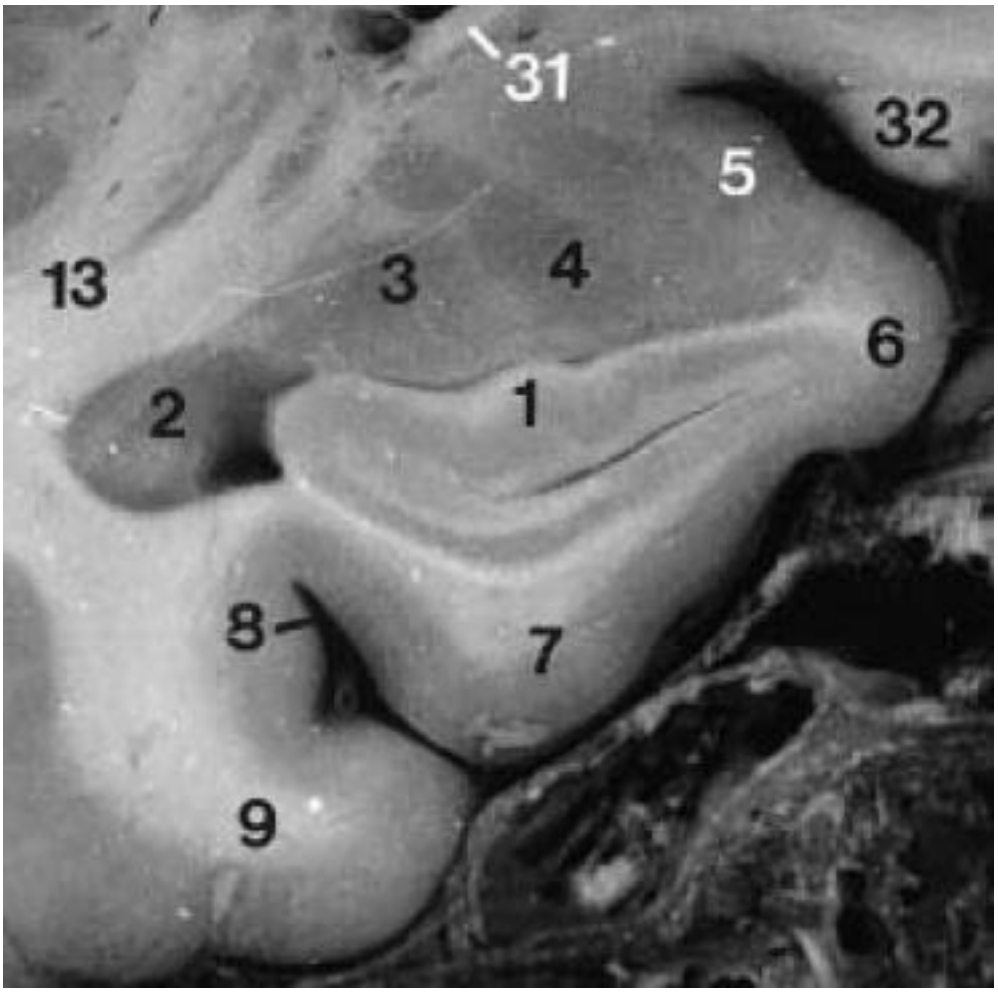


Figure 1.7: Anatomical section of the HC and its surrounding structures
1. Hippocampal head, 2. Lateral ventricle (inferior temporal horn),
3-5. Amygdala: 3. Lateral nucleus, 4. Basal nucleus, 5. Cortical nucleus
6. Ambient gyrus, 7. Parahippocampal gyrus, 9. Fusiform gyrus, 13. Temporal stem, 31. Anterior commissure (lateral part), 32. Optic tract
(Duvernoy, 2005) (Appendix B)

1.2.6 Connections to Other Structures

In the direct pathway, signals originally from the visual system directly reach the apical dendrites of CA1 pyramidal neurons from the entorhinal cortex via the perforant path through the stratum lacunosum. These pyramidal neurons then send signals via the axons on their basal side, which project to the Sub, in turn projecting to the entorhinal area (Duvernoy, 2005) (see Figure A.3 in Appendix A). This path ultimately connects to the inferior temporal association cortex, which is connected with the inferior visual system. The inferior visual system is responsible for the recognition and description of objects, which implies that this path is involved with semantic memory of the HC (Duvernoy, 2005) (see Figure A.4 in Appendix A).

Another pathway originates in a large cortical area in the temporal and occipital cortices around and including the posterior parietal association cortex (see Figure A.6 in Appendix A) and eventually travels through a path called the polysynaptic intrahippocampal pathway through the HC. The polysynaptic pathway originates at the connection from the entorhinal cortex to the DG via glutamatergic excitatory perforant fibres passing through the Sub (see Figure A.5 in Appendix A). The perforant path's fibres, along with the mossy fibres of the granular cells that they are afferent to and the Schaffer collaterals (projecting from the CA3 to the CA1), form a unidirectional three-link chain unique to the cortex (Braitenberg & Schuz, 1991). The polysynaptic pathway is more complex than the direct pathway, as it involves many neurons in different sub-regions of the HC. The fibres of this pathway cross the hippocampal sulcus and travel through

the stratum moleculare of the DG to connect with the apical dendrites of the granular neurons (Cerbone et al., 1993). The granular neurons' mossy fibres, also with glutamatergic outputs, cross the polymorphic layer to stimulate the CA3 and CA4. The CA3 and CA4 send signals through their basal axons in the alveus to both the CA1 neurons and the fimbria. Their connections to the CA1 project via Schaffer collaterals to the apical dendrites in the strata radiatum and lacunosum. Aside from sending more projections to the fimbria through its axons in the alveus, the CA1 also produces glutamatergic collaterals to the Sub. The Sub's pyramidal neurons, finally, send the remainder of the signals through the alveus to the fimbria. At the end of the intrahippocampal pathway, the fimbria extends to the crus, or "leg" of the fornix, to later reach the anterior thalamic nucleus (to targets such as the intralaminar nuclei and hypothalamus) and then to various parts of the cingulate cortex (see Figure A.6 in Appendix A) (Duvernoy, 2005; Teyler & DiScenna, 1984).

1.3 Magnetic Resonance Imaging of the Hippocampus

Traditionally, neuroscientists could only obtain information on the human brain surgically, either in post-mortem studies, which could not provide information on the ongoing progression of changes in any individual *per se*, or in *in vivo* neurosurgery, which could not look into any brain structures without damaging them in a live subject. The advent of neuroimaging adds tremendously to our knowledge of the human brain by allowing us to visualize many of these structures non-invasively *in vivo*. The past few decades have seen a dramatic rise

in the development of brain imaging techniques for visualizing brain structure (e.g. computed tomography (CT) or magnetic resonance imaging (MRI)) and activity (e.g. single photon emission computed tomography (SPECT), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (Andreasen, 1988)).

Thanks to the non-invasiveness of MRI studies, which lack ionizing radiation (used in CT scans), MRI has become an increasingly preferred method for the study of many anatomical features in the brain, including the HC (Geuze et al., 2005a). MRI methods have been shown to be very sensitive, precise, and reproducible when quantifying hippocampal volumetrics (Jack et al., 1990; Pantel et al., 2000), and have been utilized in recent decades to study many disorders.

Protocols in the literature include many different sequences for acquiring images, including the three-dimensional magnetization prepared rapid acquisition gradient echo (3D-MPRAGE) and the fast spin echo (FSE) sequence. The 3D-MPRAGE sequence uses an inversion pulse followed by an inversion recovery time (TI) to yield image contrast based on the longitudinal relaxation time (T_1) with a short repetition time (T_R). When optimized, it can yield relatively good white to grey matter contrast-to-noise ratios and is favoured over the steady-state gradient-recalled echo (GRE) sequence (Deichmann et al., 2000). The FSE sequence uses spin echo refocusing to minimize signal loss and allows for a contrast no worse than conventional spin echo (SE) sequences while taking a fraction of their time, allowing the extra time to be used to acquire higher spatial resolution images with excellent signal-to-noise ratios (SNRs) (Jackson et al.,

1997). This FSE method can produce excellent grey-white matter contrast based on differences in transverse relaxation times (T_2). Although there is good reliability between scanners with field strengths of 0.5 Tesla (T) and 1.5T using T_2 weighted imaging (Bartzokis et al., 1993a), T_2 relaxation times and quality differ between the two magnet strengths (Bartzokis et al., 1993b). A comparison of measurements between the 1.5T and 4T also showed that imaging at a higher resolution allowed for superior visualization of hippocampal detail (Mueller et al., 2007) at a higher field.

Less than two decades ago, studies of the HC used slices 5 millimetres (mm) thick, allowing for very few slices per HC (Geuze et al., 2005a; Jack, 1994), but now, slices thinner than 1.5 mm are obtainable thanks to advances in MRI technology that have allowed for better resolution and faster scanning to provide the extra time to be used in obtaining more slices. Even though thicker slices may not result in systematically biased measurements and, through a fewer total number of slices, shorten the time required for manual tracing, the thinner slices reduce the error potentially due to single mistakes made on individual slices (Laakso et al., 1997).

Protocols for tracing the HC have also been constantly evolving. For early studies, researchers measured only the body of the HC as the easiest location to trace and used its volume with the assumption that it was a representative measure of total hippocampal volume (Bremner et al., 1995; Kaye et al., 1997; Kim et al., 1994). Other studies' measurements have included the hippocampal tail along with the body but not the head (Ashtari et al., 1991; O'Driscoll et al., 2001), or the

hippocampal head and body (Jack et al., 1989). Due to its proximity, the amygdala has often been included in measurements of the hippocampal head (Shenton et al., 1992). Even acquiring the image at a different angle can result in statistically significant differences in volumetric measurements (Hasboun et al., 1996). Protocols also vary in their inclusion of diagrams to support descriptions of borders defining the HC (Geuze et al., 2005a; Malykhin et al., 2007), which may be necessary due to the difficulty of separating the HC from the amygdala, especially under lower resolutions (Soinien et al., 1994; Watson et al., 1992). Finally, other studies included the entire HC in their measurements but leave it undivided (Cook et al., 1992; Soininen et al., 1994). More recent studies provided the volumetric protocols for all hippocampal subregions (Malykhin et al., 2007).

Consistency among measurements in each study is necessary to obtain valid results regarding hippocampal differences between scans, especially if a novel protocol is used. One important factor in the reproducibility and accuracy of measurements is the consistency of individuals performing said measurements, measured by intra-rater reliability measurements. Studies have demonstrated that good intra-rater consistency can be achieved for hippocampal measurements (Jack et al., 1990), but even when the same protocols with the same anatomical criterion are used, inter-rater reliability, or reliability of measurements between observers, is more difficult to achieve (Achten et al., 1998). Unfortunately, despite its importance, many protocols did not include reliability tests (Geuze et al., 2005a).

Since the size of the ventricles has been known to correlate with the volume of cranial structures in normal adults, normalizing the volumes of brain

structures to control for intersubject variations seems to be appropriate (Synek & Reuben, 1976; Zatz & Jernigan, 1983) to minimize the assignment of larger structural sizes to individuals with naturally larger cranial volumes. Although introducing another variable to correct for head or brain size variations may lower reliability (Arndt et al., 1991), it often permits better criterion validity, allowing for better association with age and diagnosis than raw, absolute volumes (Mathalon et al., 1993). While some studies do not use any correction factors (Geuze et al., 2005a), others have normalized their hippocampal volumes to intracranial volumes (ICVs) (Jack et al., 1992) or whole brain volumes (Soininen et al., 1994). A few studies have used a regressional formula to correct for this as well (Colchester et al., 2001; Jack et al., 1989; Kopelman et al., 2001).

Although some automatic segmentation methods are constantly being developed based on existing knowledge of hippocampal anatomy (Cardenas et al., 2003; Gosche et al., 2001; Hu et al., 2011), most protocols have used manual tracing on hippocampal volumetrics, while the protocols using automatic techniques such as thresholding and region growing still use them in conjunction with manual tracing (Geuze et al., 2005a; 2005b). Even current studies using protocols involving automatic segmentations sometimes use them in conjunction with manual tracing to verify measurements (Doring et al., 2011). Due to the variability of the shape of HC, possibly leading to only some of the subjects' hippocampi being recognized by an automatic method, manual tracing is still commonly used among researchers as the gold standard of volumetric MRI. This is true especially when the subfields of the HC are measured, as even recent

automatic methods have only been at best able to measure the more uniform regions of the HC, such as the hippocampal body, rather than its entirety (Yushkevich et al., 2010).

A major limitation of hippocampal 1.5T MRI studies is that they have not resolved the inner structure of the HC (Geuze et al., 2005a). More recent studies have aimed to improve the spatial resolution of hippocampal MRI by using gains in SNR at high fields: 7.0 – 14.1T in *ex vivo* studies and 3.0 – 7.0T *in vivo* (Malykhin et al., 2010a). *Ex vivo* approaches have included magnetic resonance microscopy (MRM) and diffusion tensor microscopy (DTM) at resolutions of to 0.06*0.06*0.3 to 0.08*0.08*0.05 mm^3 (1-3 nanolitres) within short sections of the HC (Fatterpekar et al., 2002; Shepherd et al., 2007) and mapping of subfields in entire hippocampi at a resolution of 12 nanolitres (Yushkevich et al., 2009), using small bore magnets and very long acquisition times in each case. Chakeres et al. (2005) examined excised hippocampi in a 80 centimetre (cm) whole-body magnet with relatively short acquisition times, but examined single slices using a transverse electromagnetic resonator coil which was narrower (12.5 cm) than suitable for *in vivo* acquisitions. MRI studies at higher fields (7T) can even allow the visualization of distinct hippocampal layers, albeit still *ex vivo* (Wieshmann et al., 1999).

For *in vivo* studies, FSE sequences can generate high resolution images with variable contrast weightings and low sensitivity to static magnetic field inhomogeneities, but their use at high fields has been limited by the high specific absorption rate (SAR) deposition caused by their high energy radiofrequency

pulses (Lebel & Wilman 2007; 2009). For this reason, *in vivo* applications to date have utilized relatively thick slices (2-5 mm), with inter-slice gaps or either interleaved or packaged acquisitions to reduce the SAR deposition (Mueller et al., 2007; 2008; Nakada et al., 2005; Thomas et al., 2008). Even with these ameliorations, only one study imaged the full longitudinal range of the HC (Theysohn et al., 2009). High resolution gradient-echo images of the HC have been obtained at 4.1T (Pan et al., 1995) and 7.0T (Theysohn et al., 2009) and with multiple signal averaging at 3.0T (van Leemput et al., 2008), but still require steps to reduce artifacts in proximity to sinuses. With the exception of studies of normal aging and Alzheimer's Disease, which examined subfield volumes within 10 mm segments of HC (Mueller et al., 2007; 2008), studies to date have either focused on feasibility, using imaging metrics and image samples (Nakada et al., 2005; Pan et al., 1995; Theysohn et al., 2009; Thomas et al., 2008), and/or qualitative reports of abnormalities in individual patients (Eriksson et al., 2008) rather than the volumetrics of the subfields. The FSE tracing methods developed by our research group have enabled the first volumetric measures of entire hippocampal subfield volumes *in vivo* (Malykhin et al., 2010a).

Chapter 2

Functions of the Hippocampus

2.1 Overview

As it is a part of the limbic system and involved in memory, cognition, and the regulation of the hypothalamic-pituitary-adrenal (HPA) axis, the HC has been extensively studied in individuals with Major Depressive Disorder (MDD), as will be discussed in this section and the next. This region has been found to be important for declarative, spatial and contextual memory formation, mood, and nociception (McEwen, 2001; Nakamura et al., 2010).

The HC interacts with a distinct system of structures involved in storing and processing specific types of memory including the amygdala, neostriatum, and cerebellum (Squire, 2004). It also is important in general cognition, spatial navigation, mood regulation, and the response to stress, as a structure that combines the links to all those functions (Bast, 2007; DeCarolis & Eisch, 2010; Fuchs & Flugge, 1998; Woollett et al., 2009). The HC helps in the recollection of past events and can even help predict events in the future (Eichenbaum & Fortin, 2009). As a part of the limbic system, it also is heavily involved in emotion and emotional behaviour (Price & Drevets, 2010). Some of these many functions will be discussed in more detail below.

2.2 Learning and Memory

Perhaps the most well-known function of the HC is learning and memory. Adult-born hippocampal neurons as the result of neurogenesis in the HC have been shown to aid in the flexibility of cognitive functions (Burghardt et al., 2012).

As described in the two pathways in *Section 1.2.6* (*Connections to Other Structures*), the HC is involved in multiple pathways responsible for the storage of semantic (memory of details about particular facts), episodic (memory of time- and location-specific events), and spatial (memory of the environment's spatial orientation) memories. The right HC is responsible for remembering locations within a spatial environment and the left HC for context-dependent episodic and autobiographical memory (Burgess et al., 2002). Although, as shown in the famous study by Scoville and Milner (1957), many of these memories are stored outside of the HC itself and the HC may not play a vital role the retrieval of some of them, the HC still plays a major role in the process of their storage. The pivotal role that the HC plays in this storage involves the conversion of newly acquired short-term memories, which can be formed without it, into long-term memories. The spatial component of these short-term memories also relies on the HC (Duvernoy, 2005). Without the HC, this conversion is substantially hindered and newly acquired short-term memories do not get converted into long-term memories, resulting in anterograde amnesia. Other findings have also shown that lesions to the HC also lead to retrograde amnesia over time, suggesting that there is still some involvement of the HC in either or both the direct storage or retrieval of long-term memories (Nadel & Moscovitch, 1997).

The HC is not homogeneous in its functionality as segregation of functions across hemispheres and even within the same HC have been observed. Aside from the aforementioned functional segregation across hemispheres in spatial and episodic memories, novel and familiar information also is segregated.

The left anterior HC is involved in the processing of novel information both relevant and irrelevant to behaviour, while the posterior HC, bilaterally, is involved in the processing of familiar information with behavioural relevance (Strange & Dolan, 1999). During memory recollection, the activity of the hippocampal head in healthy individuals also increases relative to baseline levels, while in the hippocampal body and tail both healthy controls and MDD patients show higher levels of activation during learning than their respectively normal levels (Milne et al., 2012).

Positive correlations between hippocampal volume and memory have been frequently reported in Alzheimer's disease, Parkinson's disease, and Mild Cognitive Impairment (MCI) (van Petten et al., 2004a), however, in healthy aging, hippocampal volumes usually have been found to correlate less highly with memory (Rodrigue & Raz, 2004; van Petten, 2004b). Although age-related atrophy in the HC might be detectable in individuals with significant cognitive decline or even in the healthy aging process, the relationship between HC structural integrity and memory functions in younger populations is not well understood.

The input from the posterior parietal association cortex that eventually reaches the HC is involved in the perception of the position of an object in space (Andersen et al., 1990; Mountcastle, 1995). This also possibly contributes to the HC's role in the memory of facts in relation to one another, or the combination of episodic memory and spatial memory (Duvernoy, 2005).

2.3 Spatial Navigation

In animal studies, lesions to the HC have been shown to impair navigation (Morris et al., 1982). Neurons inside the HC create a cognitive map of the surroundings by exhibiting location-specific firing (O'Keefe & Dostrovsky, 1971). Food-storing non-human species have been shown to have larger hippocampal volumes compared to those who do not, and perform better on tasks that involve memory persistence (Biegler et al., 2001; Volman et al., 1997).

fMRI assesses activity of the brain by measuring oxygen delivery to its various regions. On fMRI, the right HC in humans has been shown to be active during the acquisition of new spatial information used for navigational tasks (Hirshhorn et al., 2011), particularly during the encoding of navigational information gained from both route (ground-level perspective) and survey (global perspective or view from the top) perspectives (Shelton & Gabrieli, 2002). This lateral activation occurs for learned environments, while for route imagery the parahippocampal gyrus is activated bilaterally (Mellet et al., 2000).

Different areas of the brain, including the parietal cortex and posterior HC, show anatomical changes in grey matter months after the learning of highly abstract information (Dragnaski et al., 2004; 2006). Cab drivers in London, England have been interesting subjects in this study due to their known ability to retain vast amounts of geographic memory of the complex city through years of course training along with years of study. These drivers must learn thousands of places of interest in London, woven within layouts of 25,000 streets, over the course of 2-4 years and pass stringent examinations by the Public Carriage Office

in order to obtain an operating license (Woollett et al., 2009). PET, another technique to image brain activity, showed with these taxi drivers that right hippocampal activation occurs also in the processing of spatial layouts formed over long periods in already well known areas (Maguire et al., 1997); the HC here is activated as a part of a vast, distributed network of active areas in the brain in response to navigation (Spiers & Maguire, 2007). On fMRI, hippocampal activation was seen in other drivers even while simply imagining themselves navigating between houses of friends in virtual reality environments (Hartley et al., 2003; Iaria et al., 2003; Maguire et al., 1998). The preferential activation of the HC along with the brain network involved in navigating between friends' homes, as opposed to a separate brain network involved in social connections, was observed in response to spatial relational processing, suggesting the preferential role of the HC in the development of a cognitive map on a large-scale space over connecting social networks (Kumaran & Maguire, 2005).

Medical doctors' training also involves a great deal of knowledge acquisition, but much of medical expertise is less spatial. A voxel-based morphometry (VBM) study on medical doctors who were IQ-controlled showed that the amount of medical expertise was not associated with any differences in grey matter volumes in the different structures of the brain, including the HC. For these doctors, the amount of medical expertise also did not correlate with grey matter volume in the HC or elsewhere (Woollett et al., 2008). Participants in the World Memory Championships, an annual competition in London, U.K. requiring participants to memorize much information not laid out in a complex spatial map,

such as numerous decks of playing cards and long lists of random digits, also were found to lack significant associations with higher IQ or any differences in brain structure relative to control participants despite the skills of memorization these contestants possess (Maguire et al., 2003). Structural changes associated with learning over many years seems to be limited to taxi drivers and their acquisition of a large, integrated complex spatial layout, such as the layout of London, U.K. (Woollett et al., 2008). These results suggest that rather than any type of memorized information, the hippocampal grey matter volume changes observed in the taxi drivers were more associated with acquired knowledge involving spatially oriented information in a complex and detailed layout.

2.4 Functions of the Hippocampal Subregions and Subfields

Selective lesion studies in rats showed that the HC is functionally subdivided along the septotemporal axis (head to tail) into dorsal and ventral regions, each associated with a distinct set of behaviours (Bannerman et al., 2004). It has been suggested that the dorsal HC, sometimes referred to as the posterior HC in primates, has a larger role in the aforementioned preferential role in certain forms of learning and memory, notably spatial learning, while the ventral HC, likewise at times referred to as the anterior HC in primates, may have a preferential role in anxiety-related behaviours (Bannerman et al., 2004). Several MRI studies have been conducted in order to replicate these findings in humans. Using fMRI, Preston et al. (2010) suggested that a gradient of content sensitivity existed from the posterior (parahippocampal) to anterior (perirhinal) MTL cortex,

with the anterior MTL cortical regions contributing to successful encoding in a domain-general manner and the posterior regions differentially contributing to successful encoding based on memory content (faces versus scenes). Chen et al. (2010a) found that the right hippocampal tail volume correlated with spatial memory, while the left hippocampal body volume was associated with delayed verbal memory. Szeszko et al. (2006) found that in healthy subjects stress correlated more strongly with the HC's anterior than posterior volume. This is consistent with another study examining hippocampal subregional volumes that reported that in young adults both hippocampal body and head volumes predicted temporal context retrieval performance while hippocampal head volume predicted spatial context retrieval (Rajah et al., 2010). Recently, Poppenk and Moscovitch (2011) reported that better recollection memory was associated with larger posterior and smaller anterior segments, as evaluated relative to the uncal apex, while overall hippocampal volume did not predict recollection memory. These studies suggest a differential specialization of various sections of the HC and therefore the importance of separating the global volume of the HC into subregions and subfields in studies of relationships between hippocampal volume and function.

Human studies of functional specialization of hippocampal subfields are limited to a few recent high resolution (< 2 mm in-plane) fMRI studies (Bakker et al., 2008; Das et al., 2011; Preston et al., 2010; Suthana et al., 2011; Yassa et al., 2011) and ultra-high resolution (< 1 mm in-plane) high field structural MRI studies (Mueller et al., 2011) that have made it possible for the first time to

demonstrate the specialization of hippocampal subfields in different memory tasks. For instance, Bakker et al. (2008) found that a pattern completion task (the ability to recall a stored memory pattern in response to a degraded observation of elements of the stored pattern) elicited a blood-oxygen-level-dependent (BOLD) response in the CA1 and Sub, and that CA3/DG activity was associated with pattern separation (the ability to keep distinct memory patterns separate). More recently, this group reported an age-related reduction in the human CA3/DG hippocampal subfields, pointing to structural and functional deficits in the perforant path along with the DG and CA3 subfields as potential contributors to the shift in memory performance throughout aging (Yassa et al., 2011). Preston et al. (2010) observed functional activation in response to both novel faces and scenes in all MTL regions. While similar percentages of voxels were sensitive to novel faces and scenes in the perirhinal and entorhinal cortices and a combined region comprising the DG and CA2-3 subfields, the parahippocampal cortex, CA1, and Sub demonstrated greater sensitivity to novel scenes than to face stimuli. Suthana et al. (2011) reported increased activity within the right CA2-3/DG subregions during encoding compared to retrieval of spatial associations. In contrast, right subicular activation increased during retrieval compared to encoding. Group differences in the activation of hippocampal subfields were also detected in Temporal Lobe Epilepsy (TLE) patients, with the DG and the anterior hippocampal region showing the greatest activation differences to controls (Das et al., 2011).

2.5 Conditions with Altered Hippocampal Volumes

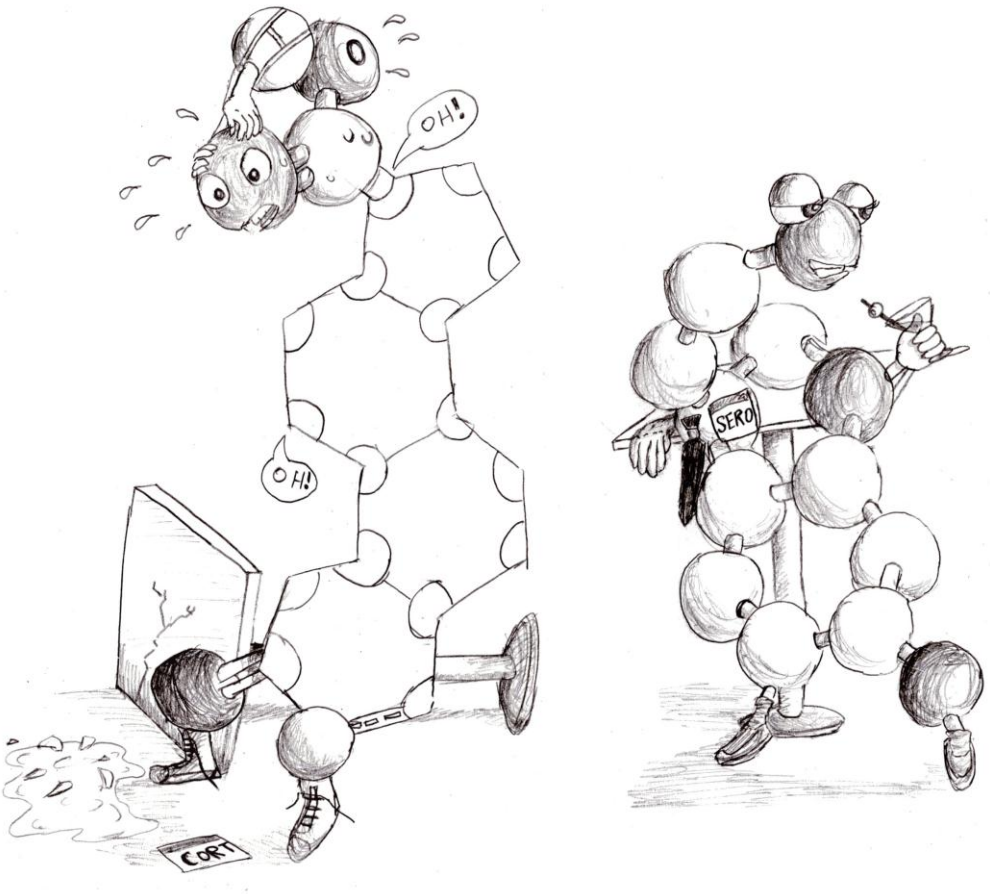
Several differing conditions have been linked in the literature with lower volumes, including normal and pathological aging (Shing et al., 2011) and different neurological and psychiatric disorders, suggesting the importance of the HC in many different functions and/or its vulnerability. Among these conditions are epilepsy (Berg et al., 2011), aging (Shing et al., 2011), Alzheimer's disease (Bralten et al., 2011; Kong et al., 2012), dementia (Yin et al., 2011), MCI (Atienza et al., 2011), traumatic brain injury (Arciniegas et al., 2001), Parkinson's disease (Messina et al., 2011), Huntington's disease (van den Bogaard et al., 2011), Cushing's disease (Tata & Anderson, 2010), schizophrenia (Adriano et al., 2011; Frith & Done, 1988), post-traumatic stress disorder (PTSD) (Zhang et al., 2011), chronic alcoholism (Sullivan et al., 1995), borderline personality disorder (Tebartz van Elst et al., 2003), obsessive-compulsive disorder (Kwon et al., 2003), antisocial personality disorder (Barkataki et al., 2006), and MDD (DeCarolis & Eisch, 2010; Holsboer, 1988).

Due to the differences in cellular compositions, functions, and molecular receptors in the different subfields in the HC, the subfields are affected differently in certain conditions. The CA1 field, along with the adjacent Sub, has been shown to be relatively more vulnerable to hypoxia than the CA3, possibly due to differences in vascular connections (Duvernoy, 2005). Kainic acid produces selective CA3 lesions, possibly due to dysfunction of mossy-pyramidal synapses here (Collins, 1986), while the DG on the other hand seems to be vulnerable to hypoglycemia (Collins, 1986). During aging, the CA1 seems to have some

neuronal loss along with other affected areas as well such as the CA4 and Sub (Mani et al., 1986; Simic et al., 1997; West, 1993). In Alzheimer's, the apical neuropil of the CA1 subfield, the region where neuronal cell bodies are located and which is one of the earliest sites of damage due to tau aggregates, undergoes localized thinning due to atrophy (Kerchner et al., 2010). In addition, the Sub consistently suffers from lesions. Other areas including the CA1 and DG are damaged sometimes, while the CA2 is more resistant to damage (Bell & Ball, 1981; Doebler et al., 1987; Haigler et al., 1985; Simic et al., 1997).

Chapter 3

Major Depressive Disorder, Stress, and the Hippocampus: from Preclinical Models of Stress to Clinical MRI Studies in MDD



3.1 Overview of Major Depressive Disorder

More than 25% of adult Americans live with at least one diagnosed mental disorder (Kessler et al., 2005a; 2005b). MDD is a commonly occurring disorder affecting significant numbers of individuals worldwide, with varying rates from country to country. It is one of the top ten causes of disability and affects from 2-5% (Murray & Lopez, 1996) to 6.7% (Kessler et al., 2005a; 2005b) of the American population. In Canada, there has been a dramatic increase in antidepressant use, with up to 3 million users in 2004, approximately 10% of the population (Picard, 2004). The diagnosis of MDD requires at least two major depressive episodes (MDEs), defined as either depressed mood and/or loss of interest or pleasure in daily activities for at least two weeks, along with a number of other symptoms causing clinically significant impairment in the individual's daily functioning, according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV). The symptoms of a MDE include depressed mood for the majority of the day, diminished interest or enjoyment in all or most activities, an unintentional significant weight change, disturbance in sleep patterns through insomnia or oversleeping, visible psychomotor retardation or agitation, fatigue or the loss of energy, feelings of worthlessness or excessive amounts of guilt, the inability to concentrate or make decisions, and recurrent thoughts of death (American Psychiatric Association, 1994).

With a median age of onset in the early 20s for most patients, MDD affects individuals of many ages, has a 40% to 55% 12-month/lifetime prevalence, and has a high rate of recurrence (people who have suffered an MDE

are likely to suffer another in the future). Although it affects both sexes and people of all marital statuses, MDD disproportionately tends to affect female and unmarried individuals. Often, MDD is also co-morbid with other psychiatric conditions such as anxiety disorders (Andrade et al., 2003; Grunhaus et al., 1994). Although considered an illness with a good prognosis, among the patients suffering from depression, 30% fail to achieve remission (Warden et al., 2007), and a diagnosis of MDD is strongly associated with elevated mortality rates (Patten et al., 2011). Additionally, depression also significantly increases the risk for cognitive impairment (Wongpakaran & Wongpakaran, 2012), with over a two-fold increase in the risk for dementia with individuals suffering from early-onset depression (Byers & Yaffe, 2011). These statistics highlight the need to research depression further in order to understand its pathophysiology, in the hope of using this knowledge for its treatment in the future.

In MDD, the HPA axis for many patients is chronically turned on, with chronically high cortisol levels in many patients (Holsboer, 2000), possibly due to dysfunction in the glucocorticoid receptor (GR) system (Barden, 1999). The negative feedback of the HPA axis to suppress cortisol does not work well in many of these subjects, as seen when dexamethasone, an artificial glucocorticoid similar to cortisol, fails to induce the body to reduce cortisol levels when administered in some subjects with MDD (Carroll, 1982). In contrast, dexamethasone usually activates the negative feedback loops in the HPA axis in controls by acting as an analogue to cortisol, reducing the upstream secretion of adrenocorticotropic hormone (ACTH) and therefore cortisol production and

secretion by the adrenal cortex (Foutoulakis et al., 2008). These increased glucocorticoid levels in stress due to HPA dysregulation is a key trait not only of MDD, but common in many neuropsychiatric disorders and illnesses with depressive symptoms (Holsboer & Ising, 2010; McEwen, 2007).

3.2 Stress and the Hypothalamic-Pituitary-Adrenal Axis

The HPA axis (see Figure 3.1) is a system consisting of multiple interacting areas in the body (mainly three endocrine glands – the hypothalamus, pituitary, and adrenal cortex) activated during chronic stress. The HPA axis allows the body to physiologically adapt to stressful events by ultimately releasing glucocorticoids, which allow the body to perform various actions to permit individuals to react to stress such as, but not limited to, mobilizing glucose via gluconeogenesis and glycolysis for the skeletal muscles, vasodilatation and vasoconstriction in different blood vessels to divert resources to cope with stress, and modifying the immune system.

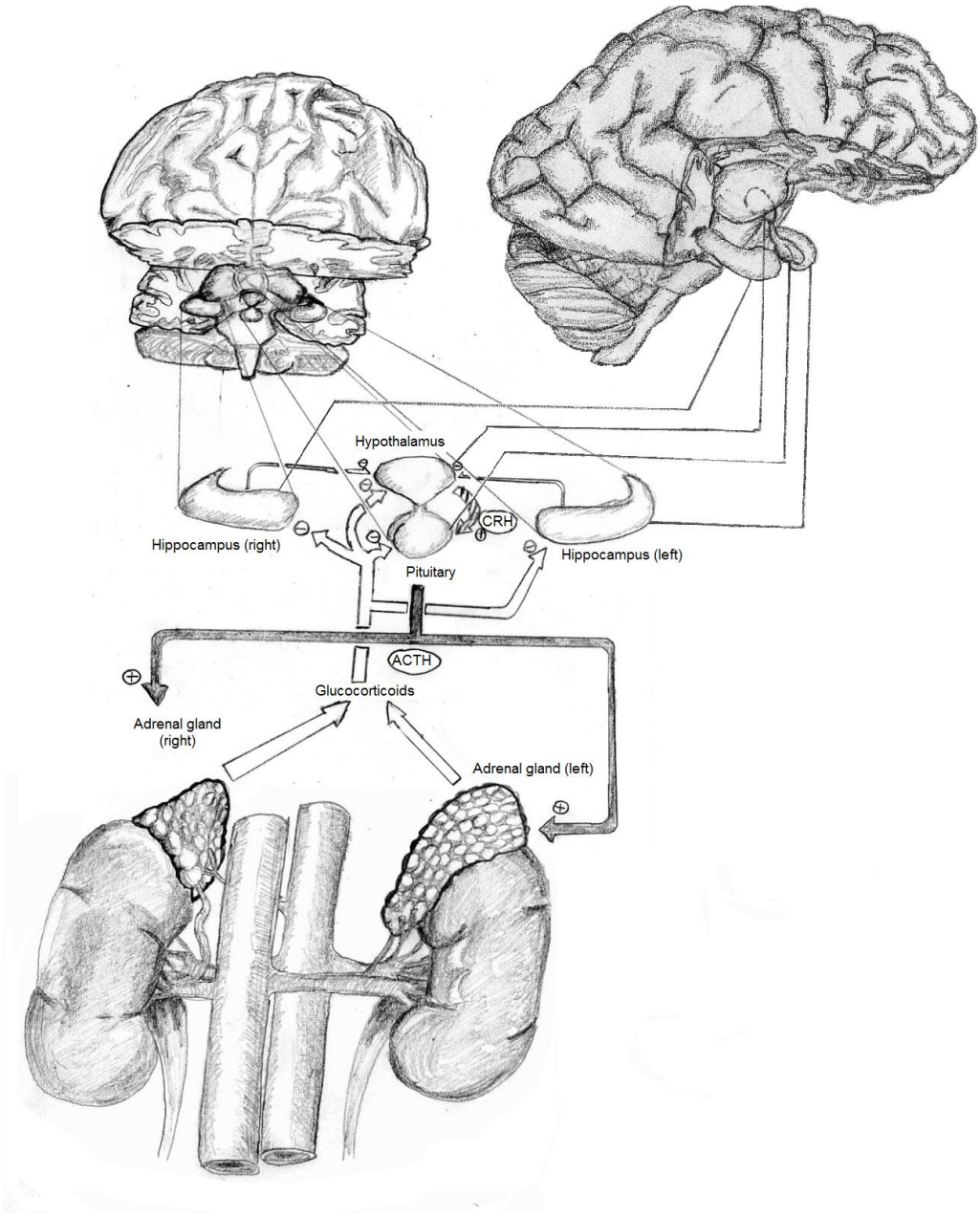


Figure 3.1: The HPA axis

This system is initially activated in response to stress when parvocellular neurosecretory neurons of the paraventricular nucleus of the hypothalamus release, among other peptide hormones, corticotrophin-releasing hormone (CRH) into blood vessels that project into the anterior pituitary via the hypothalamo-pituitary portal system. CRH reaches the anterior pituitary, signaling it to release ACTH, also known as corticotrophin, which increases the production and release of glucocorticoids, a class of corticosteroid molecules (cortisol in humans, corticosterone in some other animals such as rodents). Glucocorticoids are lipophilic hormones produced mainly in the adrenal cortex, an endocrine structure superior to both kidneys. These hormones can cross cell membranes to bind to receptors in the nucleus, sometimes inducing long-term changes in the transcription of genes. These hormones can bind to both GRs and mineralocorticoid receptors (MRs) in the nucleus, the latter due to the glucocorticoids' structural similarities to mineralocorticoids (De Kloet et al., 1998). Glucocorticoids themselves are involved in a negative feedback loop, inhibiting the hypothalamic release of CRH and also the release of ACTH from the anterior pituitary. They are released to perform many roles following stress in conjunction with other hormones such as epinephrine and norepinephrine to allow the body to mobilize metabolic resources through mechanisms such as gluconeogenesis and divert them toward the muscles in response to stress via actions such as selectively manipulating blood flow through vasodilatation and vasoconstriction. Adrenaline (epinephrine) and noradrenaline (norepinephrine) (tyrosine derivatives responsible for the more acute stress responses such as "fight

or flight", a system used to activate certain parts of the body for defense against an external agent to promote survival (Ranabir & Reetu, 2011) and to divert resources away from parasympathetic functions such as digestion in the gastrointestinal tract) levels become elevated in response to internally or externally caused disturbances of bodily functions may be interpreted by the brain as stress. Glucocorticoid levels are also increased in these stressful situations and trigger similar physiological responses (glucocorticoids are responsible for the more chronic stress responses such as decreases in the amount of resources related to long-term development and adjustments to the immune system); some responses such as gluconeogenesis are triggered by both epinephrine / norepinephrine and glucocorticoids. Epinephrine and norepinephrine released from the adrenal medulla are usually part of a more rapid and acute response to stress whereas glucocorticoids are part of a chronic or slower response. Aside from being released in response to obvious sources of stress such as a dramatic fight-or-flight response, glucocorticoids are also released periodically to control circadian rhythms which regulate metabolic, cardiovascular, and immune functions, potentially along with other biological clocks daily (Son et al., 2011).

In addition to the neuronal connections linking it to other parts of the central nervous system, the HC is also closely integrated with the endocrine system. In fact, it was the first receptor-expressing target of steroid hormones from the adrenal cortex that was found in the higher brain centres (McEwen et al., 1968). In the brain, MRs are enriched in many limbic regions – including all hippocampal subfields (along with the central amygdala, lateral septum and the

brainstem's motor nuclei); these receptors are activated by low levels of glucocorticoids (Joels, 2008). GRs are enriched in the CA1 area and the DG (together with the hypothalamic paraventricular nucleus), and despite their name, these receptors have an affinity for glucocorticoids that's ten-fold lower than the MRs, so they are typically only activated after stress or at high secretion periods during the circadian rhythm cycle (Klerman et al., 2002), in contrast to the MRs, which are normally active at rest. These properties allow the body to switch between two states: when both the GRs and MRs are activated (e.g. during stress), and when only the MRs are activated (e.g. during rest in a trough in the circadian cycle) (Joels, 2008). These receptors then in turn allow the adrenal steroids to change the excitability of hippocampal neurons by tuning the long-term potentiation (Pavlides et al., 1994; 1995a; 1995b) and primed burst potentiation (Diamond et al., 1988; 1992), in turn affecting the functions of these neurons such as memory.

Between individuals, HPA activation will vary, but in most healthy individuals, as previously mentioned, components of the HPA axis inhibit each other via negative feedback to allow the system to turn itself off when not used. This termination occurs as adrenal steroids bind to the aforementioned MRs and GRs in the pituitary, hypothalamus, and limbic system (including the HC and amygdala) (Barden, 2004). An ideal amount of glucocorticoids required for a healthy homeostatic balance exists where a large deviation to either extreme could result in pathology, similar to corticosterone's effects on burst potentiation (Diamond et al., 1992). This creates a hypothetical U-shaped curve (Sapolsky,

1997) for a healthy homeostatic system which has its peak representing the optimal response and neural survival rate created with an ideal concentration of circulating glucocorticoids. A potential mechanism for this to occur is with multiple receptors with differential binding affinities to glucocorticoids. The HC contains multiple receptors for these molecules, with the optimal response generated when a select group of receptors is activated while the rest are not (Sapolsky, 1997). Glucocorticoids are part of a stress response initiated through two-way communication between the brain and various organs regulating physiological and behavioural responses. The system is potentially adaptive, but long-term activation of it under chronic stress causes dysregulation that can be maladaptive. The adaptive part comes from the short-run restoration of allostasis, an active maintenance of homeostasis under stress, while the maladaptive part comes from allostatic load, the wear-and-tear caused on the body by chronic stress constantly putting pressure on allostasis to reach a state of homeostasis that constantly differs from the body. This glucocorticoid-mediated allostasis is achieved through dendritic remodelling and neuronal death in the limbic system, including the HC (McEwen & Gianaros, 2010).

3.3 Preclinical Models of Stress and Effects of Stress on the Hippocampus

Since depression in humans is often related to various stressful life events (such as childhood abuse and trauma, which are risk factors for depression), animal models of depression where animals are exposed to various paradigms involving chronic stress have been used to study changes in hippocampi. Additionally, some MDD symptoms resemble traits seen in animal models under chronic stress, making chronic stress a model of depression in preclinical studies. The glucocorticoids cause atrophy of hippocampal neurons' dendrites, neuronal death, and increased susceptibility to damage from other insults such as strokes or seizures (Sapolsky, 1996). Among the rodent models of depression and PTSD are models simulating chronic unpredictable stress, social defeat, and social isolation. When experienced without control and predictability over the situation, repeated stress has been shown to result in the prominent and specific altering of dendrite and spine morphology and inhibition of adult neurogenesis, along with inappropriate neuronal responses in response to brief exposure to stress (Joels et al., 2007). In these models, hippocampal neurogenesis is abated specifically in certain steps rather than homogenously throughout the entire process, particularly at the level of the type-2 amplifying progenitor cells (progenitors without axons or dendrites). The maturation into and the survival of mature neurons are shown to decrease *in vivo*, while *in vitro*, the proliferation of neuronal cells is decreased (DeCarolis & Eisch, 2010).

The HC has a dense distribution of receptors for stress hormones including glucocorticoids (Mirescu & Gould, 2006a). As stated earlier, stress activates the HPA axis, resulting in the release of corticosteroid hormones. Preclinical studies predict many mechanisms linked to the hyper-concentration of glucocorticoids in humans that might cause damage to the HC in MDD, ultimately having large effects on the architecture of the HC as a whole. These mechanisms include dendritic retraction (Czeh & Lucassen, 2007), neuronal death, and suppressed neurogenesis (Duman, 2004). Glucocorticoids originate from the stress-activated HPA axis, which is consistently overactive in many MDD subjects as seen repeatedly in studies showing increased basal cortisol levels, impairments in dexamethasone suppression, and exaggerated responses to the exposure of combined dexamethasone and CRH (Barden, 2004). As with the stress models discussed previously, while the normal, transient activation of the HPA axis in response to an acutely stressful circumstance is key to an optimal response, constant hyperactivation of the axis can be maladaptive, eventually risking the continuation into a diseased state (Joels, 2008; Krugers et al., 2000). These effects presumably result in the reduced hippocampal volumes observed in people with MDD both *in vivo* and *ex vivo* (Stockmeier et al., 2004). For a normal stress response's endocrine and behavioural aspects, newly generated hippocampal neurons in adults are needed (Jankord & Herman, 2008), along with neurons in the CA3 region; the removal of the latter disrupts spatial memory retrieval and dysregulates HPA axis activity, increasing plasma levels of ACTH and glucocorticoids (Roozendaal et al., 2001).

The reversible retraction of the dendritic tree that occurs primarily in the CA3 subfield and is caused by stress (McLaughlin et al., 2007) occurs with an accelerated rate of cell loss with increased glucocorticoid levels. This takes place along with a loss of GRs, and in chronic depression, of glucocorticoid-concentrating neurons, which also occurs in the HC during aging (Sapolsky et al., 1985). As the HC also regulates glucocorticoid levels, a loss in GRs due to damaging glucocorticoids could also create a cycle of glucocorticoid dysregulation and damage. Chronic stress for weeks also induces atrophy in the apical (but not basal) dendrites of the CA3 pyramidal neurons in the HC (Watanabe et al., 1992). Chronic restraint stress every day for 3 weeks has been shown to cause dendritic atrophy, retraction, and simplification in this subregion (McEwen, 1999). As glucocorticoids bind directly to GRs and MRs on pyramidal neurons and also to many other targets in indirect pathways, eventually reaching the CA3, these hormones, along with serotonin (also known as 5 - hydroxytryptamine (5-HT), a monoamine neurotransmitter that plays an important role in depression) and excitatory amino acids such as glutamate contribute to N-Methyl-D-aspartic acid (NMDA) receptor activity. Mossy fibres from the DG also are either directly connected via excitatory inputs to the CA3 pyramidal neurons or indirectly connected via inhibitory interneurons in the hilus synapsing onto GABA-benzodiazepine (GABA-BZ) receptors. These excitatory and inhibitory inputs are thought to normally be at a balance, as both over-reduced inhibitory activity or abnormally increased excitatory activity could contribute to atrophy (see Figure 3.2) (McEwen, 1999).

The hippocampal CA3 pyramidal cells have also been found to be most susceptible to neuronal damage and cell loss associated with prolonged social stress and glucocorticoid overexposure, in both rats and primates (Sapolsky, 2000). The total CA3 volume in the rat HC has been reported to be significantly reduced following stress, and even after a three-week recovery period the volume did not return to control values (Joels et al., 2004).

Reversible retraction of the dendritic tree occurs with some of the pyramidal neurons in the CA1 (Czeh & Lucassen, 2007). Pyramidal neurons in the CA1 of rats were observed to atrophy following treatment with corticosterone (the rat's stress glucocorticoid) (Sousa et al., 2000). NMDA glutamate receptors, which respond to estrogen, are also thought to be involved in the atrophy of CA1 neurons, as blocking them blocks atrophy (Gazzaley et al., 1996; Weiland, 1992). Biomarkers of hippocampal aging, such as the increased excitability of CA1 pyramidal neurons, are also accelerated by long-term stress (Kerr et al., 1991).

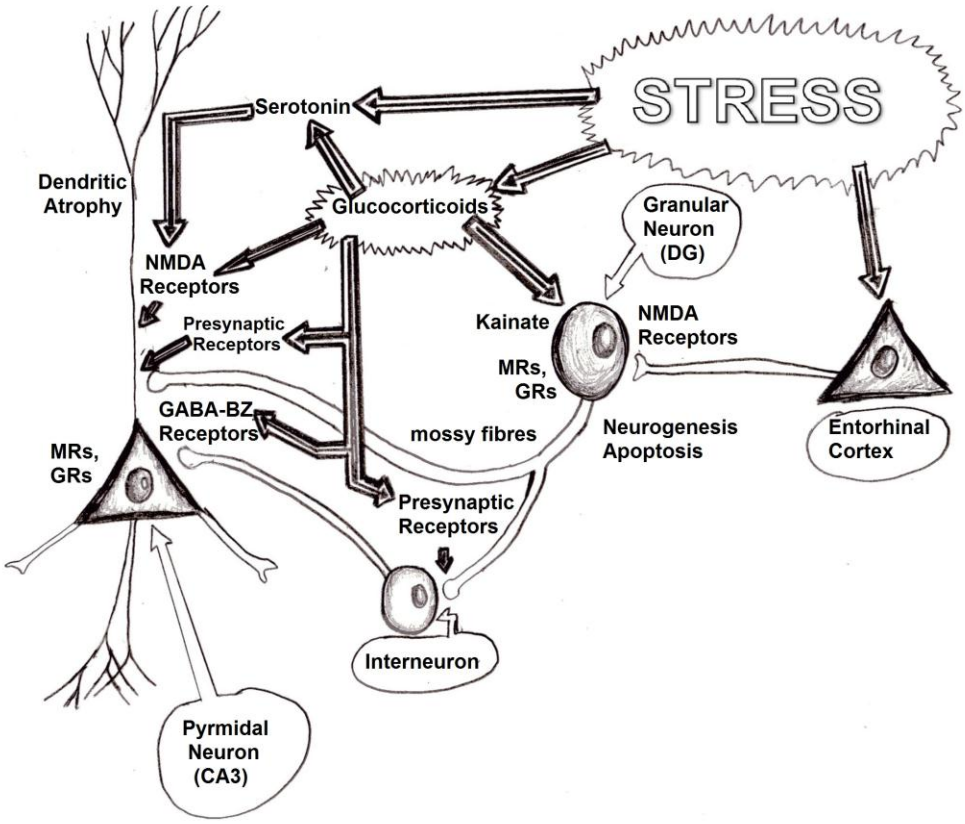


Figure 3.2: Neurotransmitter and glucocorticoid action in regulating atrophy, neurogenesis, and apoptosis in the DG and CA3 regions. MRs and GRs among other receptors in these subfields interact with glucocorticoids released by stress. Granule neurons, which are replaced in adulthood, connect via mossy fibres to the CA3 and to inhibitory interneurons in the hilus (synapsing onto GABA-BZ receptors on the CA3), providing both excitatory and inhibitory inputs. NMDA receptors could be triggered by glucocorticoids, serotonin, and/or excitatory amino acids such as glutamate, which are all present during stress to provide excitatory inputs to the CA3. The entorhinal cortex also sends excitatory signals through NMDA receptors to the DG to regulate rates of neurogenesis and apoptosis (McEwen, 1999)

Along with excitatory amino acid neurotransmitters such as glutamate, adrenal steroids such as glucocorticoids could also cause reversible stress-induced atrophy (Magarinos et al., 1996). All of these activities at abnormal rates could result in dendritic atrophy, which was observed in the CA3 region of chronically stressed rats (Sousa et al., 2000) and of tree shrews even with the absence of a decrease in neuronal count (Vollmann-Honsdorf et al., 1997).

Decreased neurogenesis seems to be involved in both anxiety and depression (Drew & Hen, 2007; Sapolsky, 2004), but as this is a relatively new research direction, evidence for the direct role of neurogenesis is limited. In mice, the inhibition of adult neurogenesis has been associated with both slower post-moderate stress recoveries to normal glucocorticoid levels and the lack of dexamethasone-induced glucocorticoid suppression. Both preclinical and postmortem studies suggest that the hippocampal subfields are differentially affected by chronic stress – following chronic stress, retraction of dendrites occurs in the CA3 region (less frequently in the CA1 or DG regions) and suppression of neurogenesis in the DG (Czeh & Lucassen, 2007).

GRs and MRs both also exist in the DG, making it directly able to respond to increased glucocorticoid levels produced by stress (McEwen, 1999). Similar mechanisms as in the CA's response to glucocorticoids also take place in the DG, but there are also many notable differences such as control over the rate of neurogenesis, which does not normally occur in the adult CA. The number of generated cells in the hilus, subgranular zone and total DG of the adult HC was significantly decreased in stressed animals (Joels et al., 2004), along with

enhanced glutamate transmission in the DG. Synaptic plasticity in both the DG and CA are severely impaired by chronic stress (Alfarez et al., 2003; Joels et al., 2004; McEwen, 2001), which can also inhibit hippocampal excitability (including primed-burst potentiation) in a path independent of endogenous opioids. The ongoing neurogenesis in the DG can be suppressed and stress-induced dendrite remodelling can occur in the CA3 of male rats and tree shrews, but these changes can be reversible if the stress does not continue past three weeks or if antidepressants are administered (McEwen, 2001). Astrocyte density has also been reported to be lower in stressed primates (Czeh et al., 2006). The ingestion of the glucocorticoid corticosterone alters serotonergic and catecholaminergic receptor activity in the DG in rats for weeks after treatment (Luine et al., 1993). Reversible retraction of the dendritic tree also occurs with granular neurons in the DG (Czeh & Lucassen, 2007). These neurons, which are replaced in adulthood and, as discussed previously, send mossy fibres to interneurons and directly to pyramidal neurons in the CA3, also have excitatory NMDA receptor inputs from the entorhinal cortex, which along with excitatory amino acids such as glutamate and adrenal steroids such as glucocorticoids regulate the neurons' rates of neurogenesis and apoptosis – making this system able to also suppress neurogenesis through acutely stressful experiences (Gould et al., 1997). Both acute and chronic forms of stress also suppress neurogenesis in the DG and increase apoptosis, while glutamate released during stress along with the glucocorticoids can lead to the reversible atrophy of the apical dendrites (in rats and tree shrews) (see Figure 3.2) (McEwen, 1999). One caveat is that these

observed changes in glucocorticoid-induced hippocampal morphology come mostly from animal experiments and have not been replicated in humans (there are no such methods to ethically replicate these types of post-mortem studies in animals where cells are visualized at precise times following controlled *in vivo* amino acid exposure), so applying this knowledge to humans requires some assumptions.

3.4 Changes in the Hippocampus in Major Depression: Clinical Studies

In clinical MRI studies, hippocampal volumes have consistently been shown to be smaller in patients with MDD compared to healthy controls (Arnone et al., 2012; Kempton et al., 2011; McKinnon et al., 2009; Videbech & Ravnkilde 2004). These reductions were found to be related to episode recurrence (MacQueen et al., 2003), duration of lifetime depression (Sheline et al., 1999) and past childhood maltreatment (Choi et al., 2009; Dannlowski et al., 2012; Frodl et al., 2010; Malykhin et al., 2010b; Vythilingam et al., 2002). Studies done more recently showed that the hippocampal subregions (head, body, and tail) are not uniformly affected in MDD (Maller et al., 2007; Malykhin et al., 2010b). Only a few MRI studies have analyzed the HC in medication-free MDD patients (Frodl et al., 2008a; MacQueen et al., 2003; Malykhin et al., 2010b; Posener et al., 2003; Vythilingam et al., 2004), while the majority of the studies included patients on antidepressant treatment (McKinnon et al., 2009; Videbech & Ravnkilde 2004).

Another limitation in these studies is the time that is required for structural changes to be detectable using volumetric MRI. For instance, increased global hippocampal volume during antidepressant treatment did not occur following a 1 year follow up, (MacQueen et al., 2003; Vythilingam et al., 2004) but only emerged following 3 years (Frodl et al., 2008a). In our recent cross-sectional study we demonstrated that MDD patients on long term antidepressant treatment (median 36 months) had larger HC body volumes than patients off antidepressants (median 12 months) (Malykhin et al., 2010b). Furthermore, lower HC volumes predicted a lower response rate to antidepressant treatment. MacQueen et al. (2008) reported that MDD patients who met the criteria for clinical remission at 8 weeks of treatment had larger pre-treatment hippocampal body/tail volumes bilaterally compared with those who were not in remission, but had no apparent difference in either the right or left hippocampal head. Furthermore, although functional and receptor measures show relatively short term changes with antidepressant treatment, PET studies in MDD revealed increased blood flow to the HC in unmedicated MDD patients and that HC metabolism, as measured via fluorodeoxyglucose using PET, was normalized after successful antidepressant treatment (Aihara et al., 2007; Videbech et al., 2002). Other studies (Drevets et al., 2007; Sheline et al., 2004) reported significant reductions in serotonin receptor binding potential for the 5-HT receptor subtypes 1A and 2A (5-HT1A/2A) in the HC that was more prominent in unmedicated MDD patients. Frodl et al. (2004) reported that MDD patients with the L/L homozygous

phenotype of the serotonin transporter gene had significantly smaller hippocampal grey matter volumes.

Along with smaller hippocampal volumes in patients with MDD compared with healthy controls, regional shape contractions have also been observed in the ambient gyrus, basal hippocampal head, posterior Sub, and dorsal HC of the hippocampi in both hemispheres (Tae et al., 2011). Another study reported that while both hippocampi were decreased in volume in a group including all of the study's depressed patients, only the right HC was shown to be smaller in a subgroup of first-episode depression patients when compared to healthy controls (Cole et al., 2010). Shape deformation in the left HC's CA3, ambient gyrus, posterior Sub, and gyrus fasciolaris also were negatively correlated with depression severity, as measured by the Hamilton Depression Rating Scale (HDRS) (Tae et al., 2011). The Sub and CA1 extending to CA2-3 also showed subregional shape deformations relative to healthy controls that were correlated with memory impairments (Cole et al., 2010).

Childhood maltreatment and abuse, itself a major risk factor for psychiatric disorders (Felitti, 2002), has been correlated with structural changes in depression, prompting many studies to investigate the relationship between them. Maltreatment and abuse has been related to reductions in the hippocampal volumes (Choi et al., 2009; Dannlowski et al., 2012; Frodl et al., 2010; Malykhin et al., 2010b; Vythilingam et al., 2002). Analysis of studies of hippocampal shape revealed that scores on the Childhood Trauma Questionnaire (CTQ), although not associated with histories of MDD or PTSD, were most strongly correlated with

maltreatment and average reductions of over 6% in the volumes of the left CA2 and CA4-DG subfields (Teicher et al., 2012). These reductions occurred along with over 4% in reductions to the left presubiculum and Sub, and other, smaller reductions in the CA1 (Teicher et al., 2012). A study of female subjects with physical and/or sexual abuse before puberty using MRI has also found that the left hippocampal volumes were 18% smaller in depressed subjects with a history of abuse than the depressed subjects without such a history and 15% smaller than healthy controls, suggesting the exacerbation of hippocampal changes by childhood abuse combined with depression.

Depression has been reported to correlate with poor performance on verbal memory, which involves tasks dependent on hippocampal performance, although no correlation was found between depression and general intellectual performance (Hickie et al., 2005; Sheline et al., 1999). Deficits in the visual memory performance in depressed subjects have also been observed (Hickie et al., 2005), as well as loss of autobiographical memory. Specifically, compared to healthy controls, MDD patients have fewer specific, positive, highly arousing and recent autobiographical memories, but more categorical autobiographical memories. These differences existed along with lower activity in the parahippocampal area and HC in MDD patients during memory recall (Young et al., 2011). The majority of the studies in MDD did not find any correlation between reductions in hippocampal volume and the severity of depression (Frodl et al., 2002; Hastings et al., 2004; Lange & Irle, 2004; Malykhin et al., 2010b). Another study did not find differences between MDD patients and controls' HC volumes, but found

smaller HC volumes in depressed patients than remitted MDD patients (Caetano et al., 2004).

3.5 Other Structural Brain Changes Associated with Major Depression

MDD is associated with fronto-limbic dysregulation through changes in amygdalar activation and prefrontal-amygdalar connectivity (Price & Drevets, 2010; Savitz & Drevets, 2009). In meta-analytic studies, anterior cingulate and orbitofrontal cortex volumes have been reported to be lower in MDD patients (Hajek et al., 2008; Hajek et al., 2009; Koolschijn et al., 2009). However, volumetric studies of the amygdala in MDD have been inconsistent in their findings (Hajek et al., 2009; Hamilton et al., 2008); some studies have found increased amygdalar volumes (Lange et al., 2004; Lorenzetti et al., 2010; Malykhin et al., 2012; Monkul et al., 2007; van Eijndhoven et al., 2009; Weniger et al., 2006), others found no difference between MDD patients and controls (Bremner et al., 2000; Mervaala et al., 2000), while others even found a volumetric decrease in patients (Caetano et al., 2004; Hasting et al., 2004; Kronenberg et al., 2009; Sheline et al., 1999; Tang et al., 2007). In contrast to the ambiguous results of the MRI volumetric studies, however, studies using both PET and fMRI have consistently found unilateral (Abercrombie et al., 1998; Drevets et al., 2002) and bilateral (Abler et al., 2007; Irwin et al., 2004; Ramel et al., 2007; Sheline et al., 2001; Siegle et al., 2002) overactivity of the amygdala in

MDD patients. These results suggest that higher neuronal activity of amygdala in MDD, along with enhanced metabolism and cerebral blood flow, might contribute to changes in the amygdala's structure.

The prefrontal cortex, a structure involved in regulating responses to stress, is linked to multiple networks involving cognitive control and mood, along with the default mode network. Altered connectivity in this structure was also observed in depressed subjects (Sheline et al., 2010). It is also involved in the stress response (Wilber et al., 2011) by being a target for glucocorticoids (Meaney & Aitken, 1985), while also showing lower levels, fewer receptors, and less binding of serotonin at multiple receptors along with lower norepinephrine receptor sites following corticosterone injection even weeks following treatment (Crayton et al., 1996; Luine et al., 1993; Takao et al., 1997). This cortical area has been shown to have lower grey matter volumes in subjects with MDD regardless of treatment or current mood state, possibly due to glial cell loss (Drevets et al., 1998). PET studies in MDD have also confirmed that MDD patients have altered cerebral blood flow in the prefrontal cortex; MDD patients also showed lower activity, as measured by glucose metabolism, in the subgenual prefrontal cortex (Drevets et al., 1997), while both lower activity level and cerebral blood flow bilaterally in the rostral prefrontal cortex were also observed (Walther et al., 2012). Decreased activation in the prefrontal cortex was observed in both medication-free and remitted subjects following a "mood challenge" involving the provocation of sadness; this was comparable to the decreased activation in medicated patients undergoing the mood challenge and acutely depressed

medication-free subjects (Gemar et al., 2007), and in contrast to never-depressed controls, who did not experience this decrease (Liotti et al., 2002; Mayberg et al., 1999). Previous studies have also shown that in response to mood provocation, unipolar depressed subjects have also shown reduced blood flow in the mediofrontal and orbitofrontal regions, areas involved in adapting and regulating stress, along with reductions of rostral cingulate blood flow compared to the lack of changes in bloodflow in these regions (Mayberg et al., 1999) and increases in the anterior cingulate activation for healthy volunteers (Liotti et al., 2002).

Lower left dorsolateral prefrontal cortex activation levels in emotional tasks in subjects with cognitive vulnerability to depression have also been observed (Zhong et al., 2011). In early-onset depression in adolescent and middle aged females, an often familial type of depression that may be genetic (Hajek et al., 2008; Hirayasu et al., 1999; Todd et al., 1993), lower left subgenual prefrontal cortex volumes have been found (Botteron et al., 2002; Drevets et al., 1997). Aside from its previously discussed associations with the HC, childhood emotional maltreatment, a risk factor for MDD (Widom et al., 2007), has also been associated with significant reductions in dorsal medial prefrontal cortex and anterior cingulate gyrus volumes (Malykhin et al., 2012; van Harmelen et al., 2010).

There are some data suggesting that other structures might also be involved in the pathophysiology of MDD, including smaller volumes for the thalamus and gyrus rectus (Kempton et al., 2011), smaller overall volumes of the frontal lobe (Kempton et al., 2011; Steingard et al., 1996), reduced frontal white

matter (Steingard et al., 2002) and enlarged lateral ventricles in MDD patients (Kempton et al., 2011).

3.6 Postmortem Studies of the Hippocampus in MDD patients

Postmortem studies have reported thinner subfield layers for the CA and DG in MDD subjects (Stockmeier et al., 2004). This was seen together with increases in the cell body densities of the DG's granule cells and glia and of all the CA regions' pyramidal neurons and glia. There were also decreases in the average size of the pyramidal neurons' cell bodies (Stockmeier et al., 2004). Lucassen et al. (2001) reported that in human post-mortem tissue, hippocampal apoptosis in MDD was a minor event and was absent from the CA3 region of the hippocampal pyramidal cell layer. However, cells undergoing apoptotic cell death were localized in the hippocampal areas CA1, CA4 and the entorhinal cortex. Responses of subjects with MDD to treatment with selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) has been shown to correlate with higher neural progenitor cell (NPC) numbers in the anterior DG (Boldrini & Arango, 2010). Experience with enriched environments, learning, and neurotrophins also were shown to increase the survival and differentiation of new NPCs (Boldrini & Arango, 2010). In MDD, lower amounts of growth factors (neurotrophins) that affect neurogenesis have been observed, which may hinder hippocampal plasticity by obstructing the formation and integration of cell

connections into brain circuits to regulate emotional responses triggered by the environment. Following treatment with antidepressants, increases in the number of NPCs and dividing cells were correlated with larger DG volumes (Boldrini et al., 2009). Mechanisms for this cell growth may involve the reversal of dendritic shrinkage, activation of anti-apoptotic proteins to enhance cell survival, and expression of brain-derived neurotrophic factor (BDNF) following treatment (Boldrini & Arango, 2010). The post-treatment BDNF expression may also be a reversal of MDD-induced changes, as MDD patients have been reported to have lower serum levels of BDNF than healthy controls and these serum BDNF levels are positively correlated with hippocampal volumes in untreated MDD subjects (Eker et al., 2010). Expression of glutamate receptor genes in the synapse was also found to be altered in post-mortem depressed subjects' hippocampi, in the DG and CA1 (Duric et al., 2012).

3.7 Changes in Hippocampal Neurogenesis in Preclinical Models of Stress

Neurogenesis is the process for the production of new neuronal cells from stem cells and progenitor cells, their precursors, in the nervous system. The only two regions in the adult human's central nervous system that have been shown with well-established, concrete evidence to undergo neurogenesis are the SGZ in the DG and the subventricular zone (SVZ). Postmortem studies have revealed progenitor cells replicating their DNA in adult human brain tissue (Eriksson et al.,

1998); the newborn neurons produced here are thought to modify the neural circuitry to provide a unique processing capacity rather than simply restoring dead granule cells (Song et al., 2012). Besides neurogenesis, the plasticity of the HC also includes the potential for much modification in the connections of neurons such as long-term synaptic potentiation and depression, dendritic remodelling, and synaptic turnover (McEwen, 2001). Neurogenesis, along with the other mechanisms of hippocampal plasticity, makes the HC very dynamic and capable of learning and memory formation. This neural versatility also exposes the HC to damage from many of the psychiatric disorders discussed above, such as PTSD (Zhang et al., 2011) and depression (DeCarolis & Eisch, 2010), and neurological disorders, such as Alzheimer's (Bralten et al., 2011) and Parkinson's diseases (Messina et al., 2011).

Many preclinical studies using animal models of depression show that in both acute and chronic stress adult hippocampal neurogenesis is suppressed (Czeh et al., 2001; 2002; Pham et al., 2003). Using mechanisms of stress and its dysregulation as a valid, comprehensive model covering many aspects of depression (Gold & Chrousos, 2002), many different forms of stress have been confirmed as potential factors can affect hippocampal neurogenesis. Adult cell proliferation can be inhibited in rodents and other mammalian species by sleep deprivation due to the elevated levels of corticosterone (Mirescu et al., 2006b). Stress also decreases the expression of BDNF, while diminished BDNF levels along with reduced levels of other neurotrophic factors can contribute to hippocampal atrophy along with the atrophy of cells in other limbic regions such

as the amygdala and prefrontal cortex (Duman & Monteggia, 2006). In male adult rats, the smell of a predator such as the odor of fox feces also acts as a stressor, inhibiting the proliferation of granule neurons (Tanapat et al., 2001). Social subordination could also lead to a lower number of new neurons in the DG in male rats, even when the amounts of cells undergoing DG cell proliferation, levels of corticosterone under basal, stressed, and stress-recovered conditions across hierarchies, and weights of the thymus, adrenal gland, and bodies are all equal (Kozorovitskiy & Gould, 2004). Mice exposed to social defeat as a stressor were also shown to have transiently fewer proliferating neurons in the subgranular zone (SGZ) as measured by the number of cells synthesizing DNA (and thus dividing) via bromodeoxyuridine (BrdU), while in the long term, DG neuronal survival was positively associated with actions taken to minimize stress such as stress-induced social avoidance (Lagace et al., 2010). Social isolation can further worsen the effects of stress by exaggerating the levels of glucocorticoids released by the stress response, inhibiting neurogenesis (Stranahan et al., 2006). In women, the effects of depression seem more prominent, as seen with the increased baseline areas under the ACTH and cortisol curves (Young et al., 2001); even more profound variations in hippocampal anatomy were linked with childhood physical and/or sexual abuse on top of depression (Vythilingam et al., 2002). This neurogenesis-suppressing stress seems to be activated by the glucocorticoid corticosterone in rodent models of decreased neurogenesis (Brummelte & Galea, 2010; Cameron & Gould, 1994; Malberg & Duman, 2003), but the inhibition of neurogenesis is absent following the introduction of a GR antagonist (Oomen et

al., 2007) or adrenalectomy (the removal of the glucocorticoid-producing adrenal glands) (Tanapat et al., 2001) even with the persistence of the stressful stimuli. Even baseline levels of glucocorticoids may inhibit neurogenesis, as adrenalectomy increases neurogenesis even in the absence of stress (Cameron & Gould, 1994). Increases in granule cell neurogenesis in the DG are also possible through exercise (Ernst et al., 2006), environmental enrichment (Nilsson et al., 1999), electroconvulsive therapy (ECT) (Madsen et al., 2000), and antidepressant medications (Boldrini et al., 2009; Malberg et al., 2000).

Not only does stress seem to hinder hippocampal neurogenesis, but evidence also exists pointing to the reverse – hippocampal neurogenesis being crucial to the recovery from stress. Inhibited neurogenesis via irradiation has been shown to block the behavioural effects of fluoxetine, an SSRI (Santarelli et al., 2003). The decrease in the rate of neurogenesis which occurs in the DG has also been suggested to play a role in the lower hippocampal volumes observed in patients with MDD (Jacobs et al., 2000). This suggests that a decrease in new neurons produced in the HC may play a role in the pathogenesis of depression, and its re-enhancement may be required for an effective antidepressant treatment (Kempermann & Kronenberg, 2003). Although the recovery from stress may depend on or at least be greatly aided by increased neurogenesis, decreased neurogenesis in one study on rodents did not seem to produce depression-like behaviours (David et al., 2010). Nevertheless, selectively removing granule cell progenitors in mice still does result in anxiety-like behaviours (Revest et al., 2009), which may be a risk factor for anxiety disorders and depression; another

study also found that neurogenesis in adults' granule cells in the DG can modulate mood and affect to serve as an effective target for the treatment of some symptoms present in depression (Danzer, 2012). Due to the role of the HC in regulating stress with its glucocorticoid receptors (ter Horst et al., 2012), stress-induced damage in hippocampal cells may induce a pathological positive feedback loop leading to further dysregulation of stress, and in turn, further stress-induced damage.

3.8 The Effect of Antidepressants on Hippocampal Neuroplasticity and Neurogenesis

Chronic, but not acute, administration of antidepressants can increase the number of dividing cells in the DG of adult rats (Czeh et al., 2001; Malberg et al., 2000) and nonhuman primates (Perera et al., 2007), and increase the number of hippocampal synapses (Chen et al., 2010b), offsetting the decrease in proliferation, atrophy, and loss of hippocampal neurons caused by stress. As previously discussed, some of the behavioural activity such as social avoidance required for the recovery from stress (Lagace et al., 2010) may also depend at least partially on neurogenesis. Serotonin receptor agonists such as agonists for the 5-HT1A receptor, a serotonin receptor found with high or very high density over the CA1 subfield of the HC and raphe nuclei (Pazos et al., 1987), have demonstrated the ability to upregulate neurogenesis (Banasr et al., 2006), while antagonists of this receptor downregulated such effects (Radley & Jacobs, 2002).

This shows the importance of the serotonergic pathways in the restoration of neurogenesis in depression, and is consistent with the mechanism of antidepressants, as many antidepressant classes such as the MAOIs, SSRIs, and TCAs work directly or indirectly to modify the synaptic activity of serotonin and sometimes norepinephrine, and consequently, neurogenesis (Santarelli et al., 2003; Wong et al., 2005), sometimes also via BDNF (Balu et al., 2008; Duman & Monteggia, 2006; Li et al., 2004). These studies hint that neurogenesis is necessary for the clinical efficacy of antidepressants, on the molecular, cellular, and behavioural levels, although there is some evidence suggesting that neurogenesis *per se* may not be required for all the antidepressant effects of medications (Holick et al., 2008; Singer et al., 2009). Several studies suggested that the antidepressant-enhanced neuroplasticity's importance may depend on some mechanisms of action of these drugs (Bessa et al., 2009; Wang et al., 2008), some possibly requiring neurogenesis while others may not (David et al., 2009; Surget et al., 2008).

Antidepressants increase the number of cells undergoing mitosis across various age groups in the DG, but this increase is lower in magnitude for lower mammals of higher age, possibly accounting for the poorer antidepressant response in the elderly (Couillard-Despres et al., 2009). In rats, stress is correlated with a reduction of neurogenesis while neurogenesis is enhanced by environmental enrichment, exercise, and antidepressants. However, compromised neurogenesis does not induce depression-like behaviour (Boldrini & Arango, 2010). Neurogenic regions of the adult rodent brain experience an increase in cell

proliferation (as detected by the administration of BrdU) in response to the administration of some antidepressants or atypical (second generation) antipsychotics, which could lead to either larger volume or mass for those areas. Those drugs also stimulate neurotrophic factor production, which could further stimulate increased neuronal survival rates or neurogenesis (Nasrallah et al., 2010).

Antidepressant drugs such as fluoxetine and imipramine have been shown to increase the proliferation of neurons *in vitro* and also to increase the number of maturing cells in MDD and PTSD *in vivo* at three stages (maturation into type-2 amplifying progenitors, post-mitotic neuroblasts, and mature neurons) while promoting the survival of mature neurons. Behavioural treatments such as exercise seem to have similar effects as antidepressants, increasing progenitor cells at the same stages and also increasing the survival of maturing cells (DeCarolis & Eisch, 2010).

Polymorphisms in the 5-HT transporter (5-HTT) gene have been found to be important in the link between early and adult stress models of depression in mice, with the HC also playing a crucial role in this interaction (Carola & Gross, 2012). Certain alleles for this gene also increase the risk for depression in humans when combined with environmental factors including childhood abuse (Caspi et al., 2003). The previously discussed studies on childhood abuse serve as important examples to show how environmental stressors can play a major role in depression, and their effects may linger from childhood into adulthood.

Hippocampal neurogenesis seems to also depend on the serotonergic neurons originating in the raphe nuclei and innervating the DG, as the rates of neurogenesis decrease with lesions in these neurons and recover following neuronal implantation (Brezun & Daszuta, 2000). This is similarly true with parallel experiments with the locus ceruleus' noradrenergic connections to the DG subfield (Kulkarni et al., 2002), suggesting a link between the serotonergic and noradrenergic actions of antidepressants and neurogenesis in the treatment of depression (Santarelli et al., 2003).

Antidepressants act through serotonergic and noradrenergic mechanisms, and ultimately reverse the adverse changes in the plasticity and volume of the HC that are caused by stress (Czeh et al., 2001). With chronic administration in adult rats, the efficacy of different classes of antidepressants (SSRIs, norepinephrine reuptake inhibitors (NERIs), and monoamine oxidase inhibitors (MAOIs)) have been shown to be positively related to the number of dividing cells in the DG, showing a link between the therapeutic effects of antidepressants and the reversal in the stress-induced decrease in proliferation, atrophy, and loss of neurons in the HC (Malberg et al., 2000). Serotonin may itself play a neuroprotective role, as its N-acetyl derivatives N-acetylserotonin (NAS) and N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC) have been shown to be potent neuroprotectants elsewhere (Shen et al., 2012). Antidepressants have also been shown to produce a recovery of the diminished BDNF levels commonly observed in depression, which along with the cyclic adenosine monophosphate response element binding (CREB) transcription factor, seem to be also be related to

neuroprotection (Chang et al., 2006); this upregulation of BDNF has been shown to play a role in the reversal of the neuronal atrophy and loss of cells caused by the BDNF shortage observed in depression (Duman & Monteggia, 2006). Bilateral human ECT is known to have antidepressant effects similar to pharmaceutical antidepressants, as demonstrated in adult nonhuman primates using electroconvulsive shock (ECS), the animal counterpart of ECT. ECS increases cell proliferation in the SGZ of the DG by increasing the proliferation of precursor cells and differentiated neurons (Perera et al., 2007). In functional tests using PET, ECT has been shown to be involved in the activity of important serotonergic pathways as well (Saijo et al., 2010).

TCAs inhibit the reuptake of norepinephrine and/or serotonin to increase their levels in the synaptic cleft and therefore activate more receptors in the postsynaptic membrane. The TCA clomipramine, a strong inhibitor of serotonin uptake, has been shown to increase the expression of four out of five genes that are underexpressed during stress (Alfonso et al., 2004) while another TCA, desipramine, a potent norepinephrine reuptake inhibitor, leads to recovery of the synapses lost during stress (Hajszan et al., 2010). TCAs can also continue to enhance neurogenesis even in 5-HT subtype 1A (5HT1A) receptor-knockout mice by acting on norepinephrine pathways as, unlike SSRIs, they inhibit the reuptake of norepinephrine into the presynaptic neuron as well (Santarelli et al., 2003).

MAOIs inhibit the mitochondrial enzyme monoamine oxidase (MAO), responsible for the presynaptic breakdown of monoamines such as serotonin and norepinephrine. Both nonselective irreversible MAOIs such as tranylcypromine

and phenelzine and reversible MAO-A inhibitors such as moclobemide have been shown to recover the BDNF levels in the brain that were lowered by chronic stress (Balu et al., 2008; Duman & Monteggia, 2006; Li et al., 2004). These drugs also promote the replication of progenitor cells, as measured by cells' synthesis of DNA.

SSRIs work at the synapse to inhibit the serotonin transporter, hindering the reuptake of serotonin into the cell to allow it to theoretically increase the duration of its activity in the synapse; examples of these drugs include citalopram and fluoxetine. This class of drugs is known to reverse the inhibition of hippocampal neurogenesis produced by stress (Wong et al., 2005) and also to reverse the behavioural effects of stress. Hippocampal volumes in PTSD have been shown to increase following treatment with sertraline, another SSRI (Bossini et al., 2007).

Serotonin-norepinephrine reuptake inhibitors (SNRIs), newer drugs than the TCAs that also inhibit the reuptake of both neurotransmitter amines, are also among the drugs used to treat MDD. *In vivo* evidence shows that the administration of duloxetine, an SNRI, results in significant volumetric increases in the HC along with increases in other structures such as the nucleus accumbens, putamen, and brainstem after just six weeks of treatment (Lai & Wu, 2011).

Selective serotonin reuptake enhancers (SSREs) have immediate mechanisms at the synapse that are opposite of those of SSRIs as they work by increasing the reuptake of serotonin into the presynaptic cell. Despite this mechanism being the reverse of that of SSRIs, SSREs have been shown to also

prevent the stress-induced atrophy in the CA3 pyramidal neurons' apical dendrites on top of stimulating neurogenesis at the DG. Tianeptine is the only currently available antidepressant of the SSRE category (Czeh et al., 2001; Magarinos et al., 1999).

Antidepressants may function by increasing the sensitivity of the HPA axis to glucocorticoids by increasing GR activity. This increase in receptor activity may increase the HPA axis' response to its own negative feedback, allowing it to eventually decrease its hormonal activity, putting an end to the exaggerated chronic stress response (Barden, 1999). A major problem with relating the neurogenesis antidepressants induce to MDD is that although adult neurogenesis has been demonstrated to be an important link to chronic stress in non-human species such as rodents, very few such studies to date have shown the same effects in humans (Balu & Lucki, 2009). Two postmortem studies have looked at the effects of antidepressants on adult human neurogenesis. One reported no effect of antidepressants on neurogenesis (Reif et al., 2006); the other found that both SSRIs and TCAs increased the number of NPCs while treatment with TCAs also increased proliferative cells (Boldrini et al., 2009).

Chapter 4

Objectives and Hypotheses

4.1 Objectives

The HC's neuroplasticity, although important in learning, memory, and spatial navigation, make it very vulnerable to stress at least partially due to its receptors for glucocorticoid stress hormones. The well-described effects of stress on the risk of developing MDD have been supported by the findings of abnormalities in the HPA axis in patients with depression.

One of the most interesting findings has been that hippocampal volume is decreased in patients with MDD; another area of growing importance is the increased understanding of the possible role of neuroplasticity in MDD. There is preclinical evidence that stress and glucocorticoids negatively impact hippocampal neuroplasticity, neuronal survival, and glial survival, while other pre-clinical studies have suggested that antidepressants have stress-protective effects on hippocampal neuroplasticity. As such effects also appear to occur in humans, if these mechanisms became better established, significant new possibilities for studying both the etiology and treatment of MDD could be unveiled.

A major research question is whether *in vivo* hippocampal subfield volumes are differentially affected by MDD or antidepressants, but this has not been tested to date due to the inadequate spatial resolution of conventional MRI. Newly developed applications on a high-field 4.7T MRI have allowed us to test this *in vivo* for the first time. With these applications available to us, the main objective of the present cross-sectional study was to investigate whether

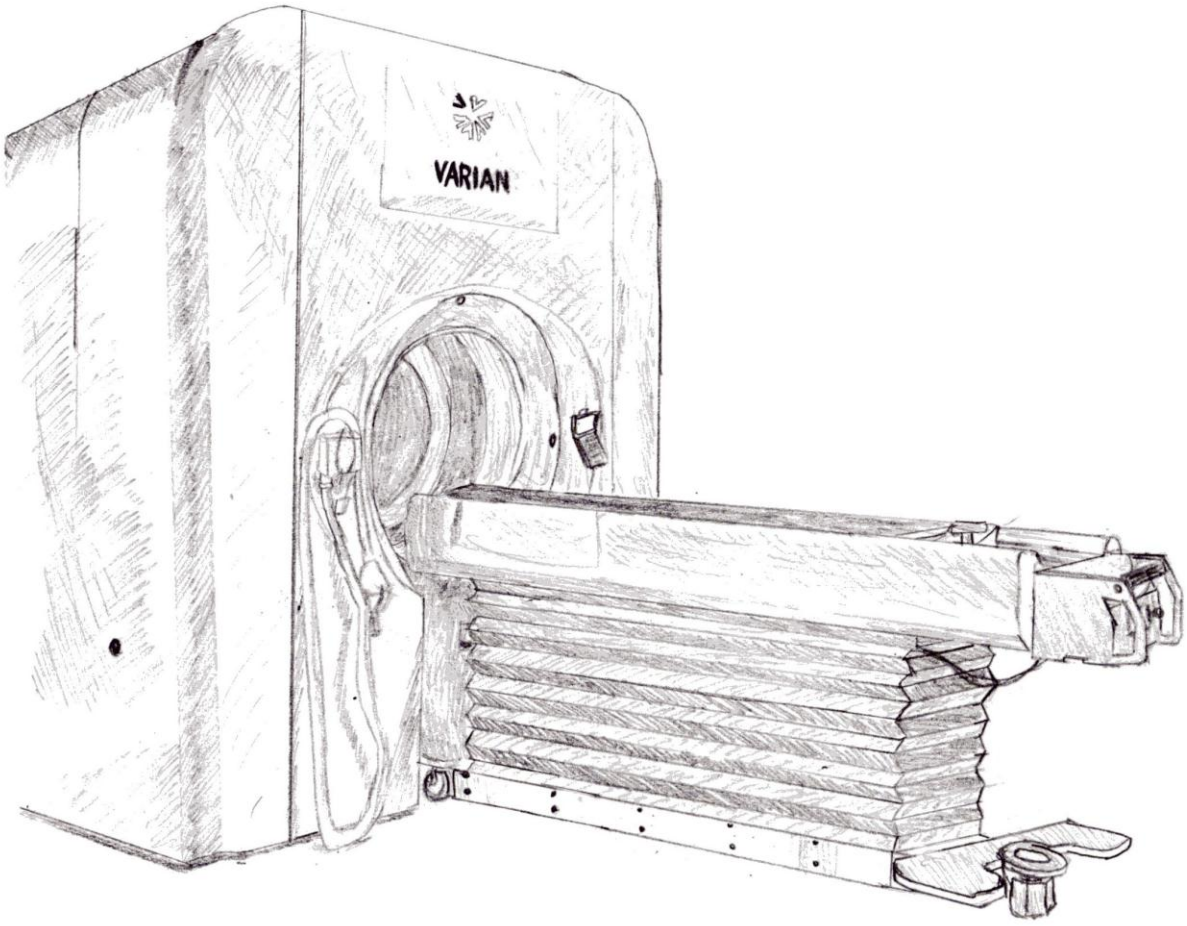
hippocampal subfields are differentially affected in MDD and whether those changes can be reversed by antidepressant treatment.

4.2 Hypotheses

Based on all the evidence that has been uncovered thus far about depression and antidepressant treatment, including the preclinical and postmortem studies discussed previously, we formulated two hypotheses regarding predicted differences in hippocampal subfields between different treatment and diagnosis groups. First, we hypothesized that the CA subfield would be smaller in MDD subjects compared to healthy controls due to its vulnerability to the stress response in the preclinical models of depression. Second, we hypothesized that the DG would be larger in medicated MDD patients and/or healthy controls compared to unmedicated MDD patients due to the aforementioned stress-suppressed and antidepressant-induced hippocampal neuroplasticity/neurogenesis.

Chapter 5

Methods



5.1 Recruitment of Subjects

5.1.1 Recruitment and Assessment

Subjects for this study were recruited through notices in the community and around the University of Alberta campus, including the university hospital. They were assessed in the outpatient psychiatry department by Dr. Nick Coupland, a clinical psychiatrist. MDD patients' symptom severity was assessed via the HDRS (Hamilton, 1960) and the Mood and Anxiety Symptoms Questionnaire (MASQ) (Watson et al., 1995a; 1995b), and subjects were included if they had moderate or severe MDEs according to the DSM-IV criteria based on a full clinical assessment and the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al., 1997).

5.1.2 Inclusion Criteria

MDD patients in the unmedicated MDD group were free of medication for at least 6 months or more, and patients in the medicated MDD group had been continuously receiving antidepressant treatment for more than 12 months. As childhood maltreatment is an important factor in depression, it was also evaluated using the CTQ. With life chat methods and case records, the age of onset, number of episodes, illness duration, and treatment history were all assessed.

The control and MDD groups were matched for age, sex, education, and ICV following the measurements of brain structures. All subjects were right handed. The study was approved by the University of Alberta Health Research Ethics Board, and each subject signed an informed consent form.

In total, 47 subjects aged between 18 and 49 were included: 9 unmedicated MDD subjects and 11 medicated MDD subjects for a total of 20 MDD subjects, along with 27 matched controls. See Table 6.1 for demographics.

5.1.3 Exclusion Criteria

1. Mild depressive episodes, psychotic or atypical features, seasonal affective disorder
2. Lifetime schizophrenia, bipolar disorder
3. Alcohol or substance dependence, anorexia nervosa
4. Antisocial or borderline personality disorder, predominantly anxiety disorder
5. Systemic corticosteroid use and lithium or anticonvulsant mood stabilizer use
6. Significant medical or neurologic disease
7. Pregnancy, obesity

To control for genetic and other inheritable factors on morphology and possible susceptibility to MDD, only controls with no lifetime or first-degree relative history of psychiatric disorders or reported psychosis were used. To control for the different ages of MDD onset, elderly subjects aged 50 years and above were not included in any groups.

Due to recruitment restrictions, our unmedicated and medicated groups each had few subjects alone. For this reason, we decided to also combine the two groups into an MDD group and additionally look for common changes, as both groups have experienced MDD (see the left-most and right-most columns on Table 6.1 for the groups following amalgamation). This way, we conducted an

analysis with a sample of 20 subjects (40 hippocampi from both hemispheres) with MDD, compared with 27 matched healthy controls. This particular analysis gave us more statistical power, as the group with the least number of subjects had a higher sample size, but did not allow us to observe the effects of medication.

5.2 MRI Data Acquisition and Analysis

5.2.1 Image Acquisition

Images were acquired using the University of Alberta's Varian Inova 4.7T scanner. Two different types of images were collected for each subject:

T2-weighted two-dimensional (2-D) FSE images were acquired with a field of view (FOV) of 20 cm * 20 cm to encompass the HC each containing 90 slices with an echo time (TE) of 39 milliseconds (ms), TR of 1.1 seconds, and total acquisition time of 13.5 minutes. The spatial resolution was 0.54 mm * 0.68 mm * 1.00 mm, which was then interpolated 2x in-plane.

T1-weighted 3-dimensional (3-D) MPRAGE images were acquired encompassing the entire brain in order to measure the ICV, later used as a covariate to control for varying brain sizes. This method used 256 slices with a TE of 5 ms, TR of 1.8 seconds, TI of 850 ms, isotropic resolution of 0.75 mm * 0.75 mm * 0.75 mm, and acquisition time of 15 minutes).

5.2.2 Contrast Adjustment

Following the scan, both FSE and 3-D MPRAGE images were converted to MINC (.mnc) files, a format compatible with DISPLAY, our image processing software (*see Section 5.3 (Image Analysis)*). As the FSE scans were obtained via T2-weighted imaging (see Figure 5.1), the FSE images' contrast was inverted for the white matter to appear white/lightest, grey matter to appear grey/darker, and for cerebrospinal fluid (CSF) to appear black/darkest for processing (see Table 5.1).

This changes the FSE images to follow similar colour schemes as the T1-weighted 3D-MPRAGE images for consistency during manual ICV/hippocampal subfield quantification later. The contrast during image processing was then finely adjusted before image analysis as discussed below.

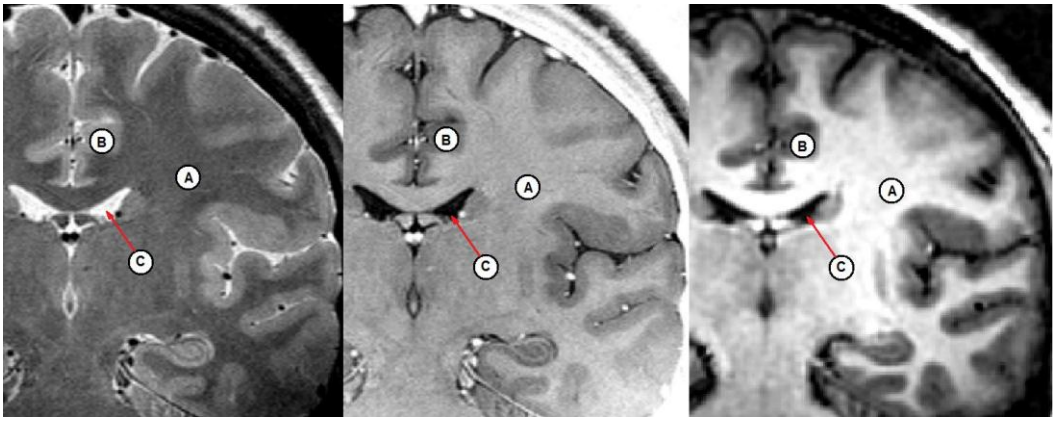


Figure 5.1: Comparisons of grey matter, white matter, and CSF as viewed from three types of images: T2-weighted FSE (left); T2-weighted FSE with inverted contrast (centre); T1-weighted 3D-MPRAGE (right)

	T2-weighted FSE	T2-weighted FSE (Inverted contrast)	T1-weighted 3-D MPRAGE
White Matter (A)	Darker	Lightest	Lighter
Grey Matter (B)	Dark	Darker	Darker
CSF (C)	Lightest	Darkest	Darkest

Table 5.1: Relative shading levels of grey matter, white matter, and CSF from the T2-weighted FSE, T2-weighted inverted FSE, and T1-weighted 3D-MPRAGE

5.3 Image Analysis

5.3.1 Image Processing and Segmentation

The images were analyzed and quantified using the interactive public domain software DISPLAY, a program developed by the Montreal Neurological Institute (MNI). Using DISPLAY, measurements of the regions of interest, including the ICV from the T1-weighted 3-D MPRAGE and the hippocampal subfields from the T2-weighted 2-D FSE were obtained. The measurements of the HC separated by hippocampal head, body, and tail were measured *in vivo* and quantified using previously published methods (Malykhin et al., 2007; 2010a) *(see Section 5.3.2 (Tracing Protocol))*. These methods included the hippocampal tail, which is neglected by some other researchers due to the complexity of the HC in that area despite its importance for accurate volumetrics (Maller et al., 2007). ICV values were calculated using an estimation obtained by tracing every tenth sagittal slice on the T1-weighted 3-D MPRAGE and multiplying the volume traced by ten, which has been shown as an accurate method that does not compromise reliability (Eritaia et al., 2000).

5.3.2 Tracing Protocol

The ICVs were measured using sagittal slices of the MPRAGE scan by tracing the ICV portion of the brain (including grey matter, white matter, and CSF) for every tenth slice (for approximately 27 slices if the slice width is 2mm) for an estimate of one-tenth of the ICV and multiplying the resulting volume by ten (see Figure 5.2).

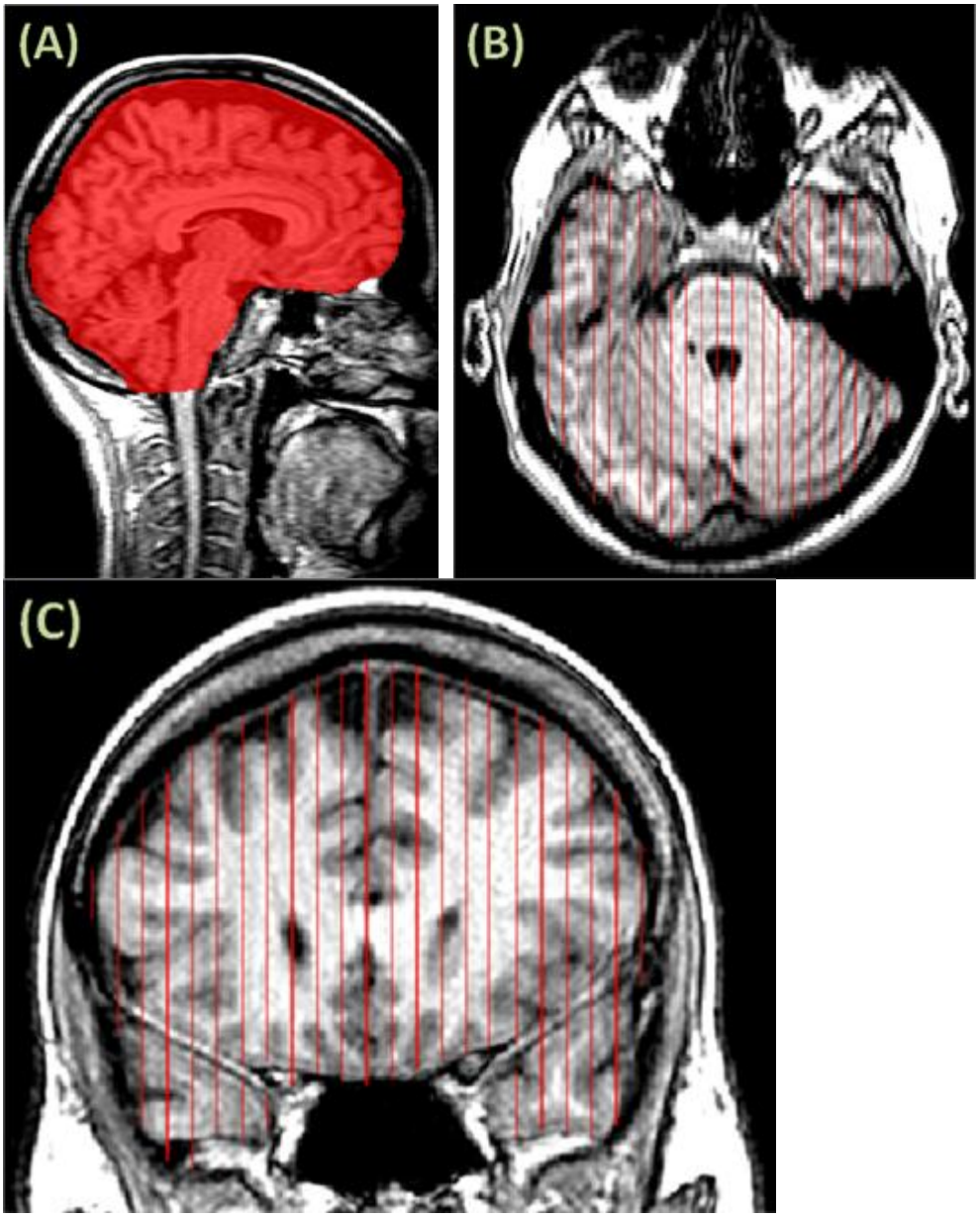


Figure 5.2: Full-brain manual segmentation of MPRAGE scans to calculate ICV values

A. in sagittal, B. axial, and C. coronal views

All hippocampal tracings were done on coronal slices of the FSE scan, for every slice (typically approximately 16 slices for the hippocampal body, 9 for the tail, 19 for the head, depending on the individual subject) on which the HC was visible.

The hippocampal body (see Figure 5.3) appears in the middle of the HC immediately anterior to the hippocampal tail and posterior to the hippocampal head. The strata lacunosum and moleculare separate the DG from the Sub and CA. The boundary of the Sub and the CA is delineated as a linear lateral-inferior extension of the medial-inferior boundary of the DG. The hippocampal body continues until its most anterior slice, the slice immediately posterior to the most posterior slice of the hippocampal head, which is described below.

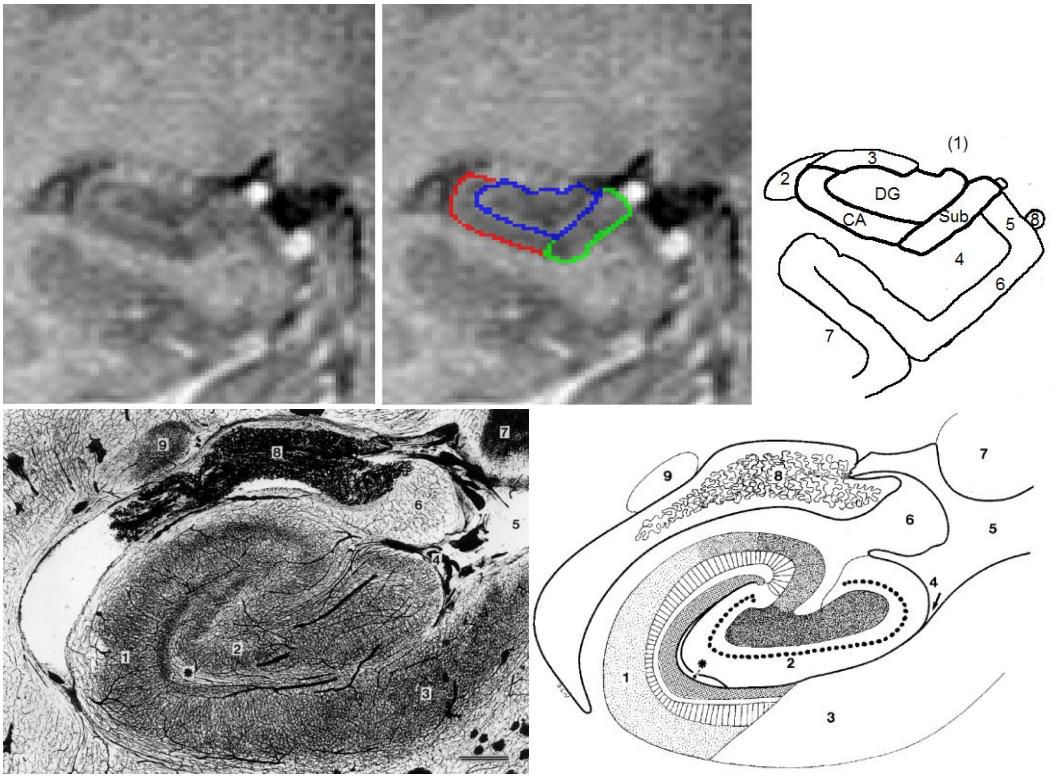


Figure 5.3: The hippocampal body

Top-left and top-centre: Inverted T2 4.7T FSE scan, without and with boundaries;

Top-right: Sketch of the boundaries shown on the scan.

(1) approximate location for the lateral geniculate body, 2. lateral ventricle (temporal horn), 3. fimbria, 4. parahippocampal gyrus, 5. presubiculum, 6. entorhinal cortex,

7. fusiform gyrus, 8. posterior cerebral artery

Bottom-left: vascular injection; Bottom-right: sketch of the boundaries.

1. CA, 2. DG, 3. Sub, 4. margo denticulatus (rounded protrusions superficial to DG), 5. transverse cerebral fissure (lateral part), 6. fimbria, 7. lateral geniculate body; 8. choroid plexuses in the lateral ventricle (temporal horn); 9. caudate nucleus

(Duvernoy, 2005) (Appendix B)

The hippocampal tail's (see Figure 5.4) most anterior slice is the more anterior of two slices (the respective properties of which may coincide to make them one slice) – either the slice of the temporal horn connecting with the rest of the lateral ventricle or the slice of the fornix appears in full profile. The hippocampal structure turns medially in each subsequently posterior slice, while the DG remains separated from the CA and the Sub by the strata lacunosum-moleculare. The Sub in the tail's most anterior slice is first fully extended along the area of the HC inferior and medial to the DG, and in the subsequent posterior slice occupies the more superior-medial half of that area. Posterior to those two slices, the Sub is discontinued (leaving only the DG and the CA).

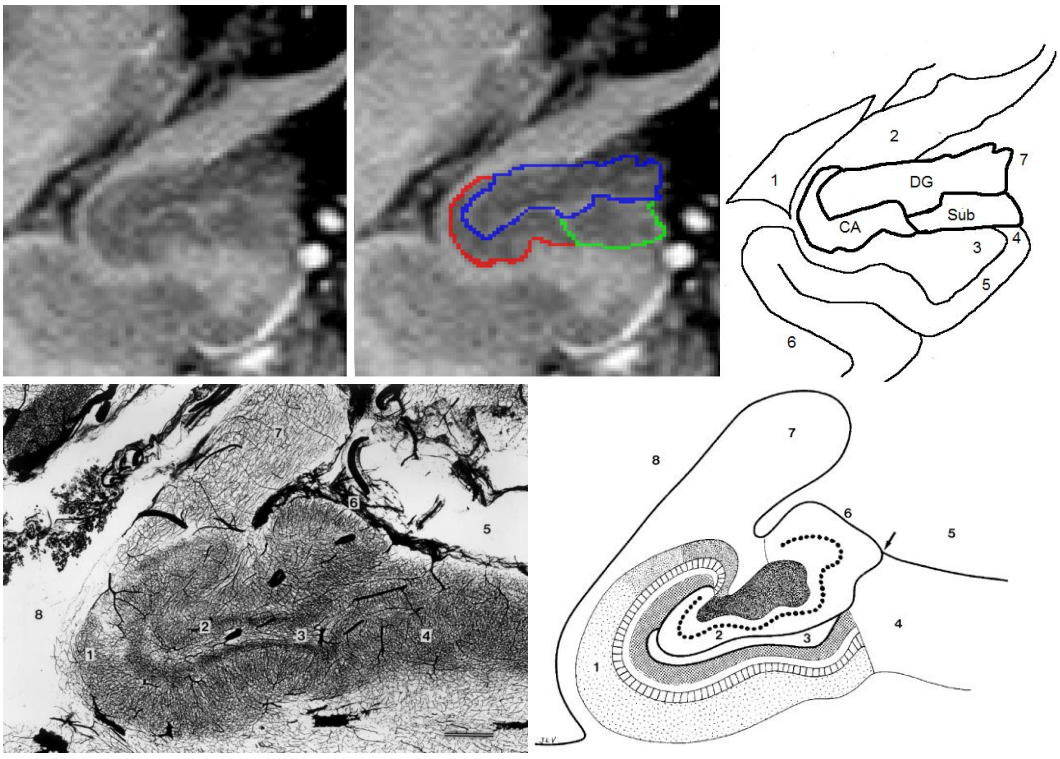


Figure 5.4: The hippocampal tail

Top-left and top-centre: Inverted T2 4.7T FSE scan, without and with boundaries;

Top-right: Sketch of the boundaries shown on the scan.

1. lateral ventricle (temporal horn), 2. fornix, 3. parahippocampal gyrus (grey matter in this area is isthmus of cingulate gyrus at more posterior slices), 4. presubiculum, 5. entorhinal cortex, 6. fusiform gyrus, 7. ambient cistern.

Bottom-left: vascular injection; Bottom-right: sketch of the boundaries 1. CA, 2. DG, 3. hippocampal sulcus (vestigial), 4. Sub,

5. transverse cerebral fissure (lateral part), 6. margo denticulatus (rounded protrusions superficial to DG), 7. fornix (crus), 8. lateral ventricle (temporal horn)

(Duvernoy, 2005) (Appendix B)

The hippocampal head (see Figure 5.5) starts with a prominent medial extension of the HC into the uncinate gyrus. The DG is still separated by the strata lacunosum and moleculare, and reaches its anterior end before the other subfields when its boundaries separate into individual digitations on its most anterior slices. The HC on its most anterior slices then becomes an increasingly smaller boundary as the amygdala takes up more of the grey matter superior to it in each subsequent slice is included as the amygdala superior to it.

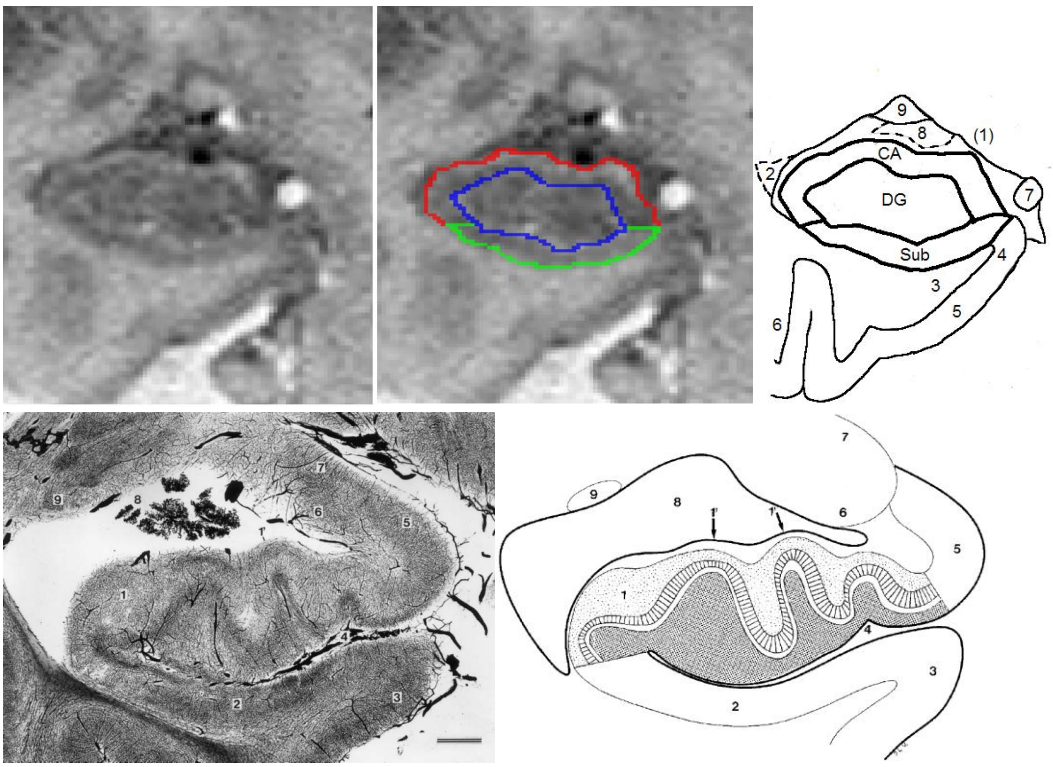


Figure 5.5: The hippocampal head

Top-Left and top-centre: Inverted T2 4.7T FSE scan, without and with boundaries;

Top-right: Sketch of the boundaries shown on the scan.

(1) approximate location for the lateral geniculate body / optic tract [more anterior], 2. lateral ventricle (temporal horn; prominence depends on individual), 3. parahippocampal gyrus, 4. presubiculum,
5. entorhinal cortex, 6. fusiform gyrus, 7. posterior cerebral artery,
8. choroid plexus, 9. Amygdala

Bottom-left: vascular injection; Bottom-right: sketch of the boundaries.

1. CA, 1'. digitationes hippocampi (internal digitations) 2. Sub,
3. parahippocampal gyrus, 4. uncal sulcus, 5. Sub in uncinate gyrus,
6. amygdala (accessory basal nucleus), 7. amygdala (cortical nucleus);
8. lateral ventricle (temporal horn); 9. caudate nucleus (tail)

(Duvernoy, 2005) (Appendix B)

Although the sagittal plane is not used during tracing itself, it can be a good plane to illustrate how the various coronal slices anatomically are situated in the HC. 3-D recreations of the HC using our tracing method (see Figure 5.6) also help map the locations of the subfields relative to each other, and correspond similarly to anatomical landmarks.

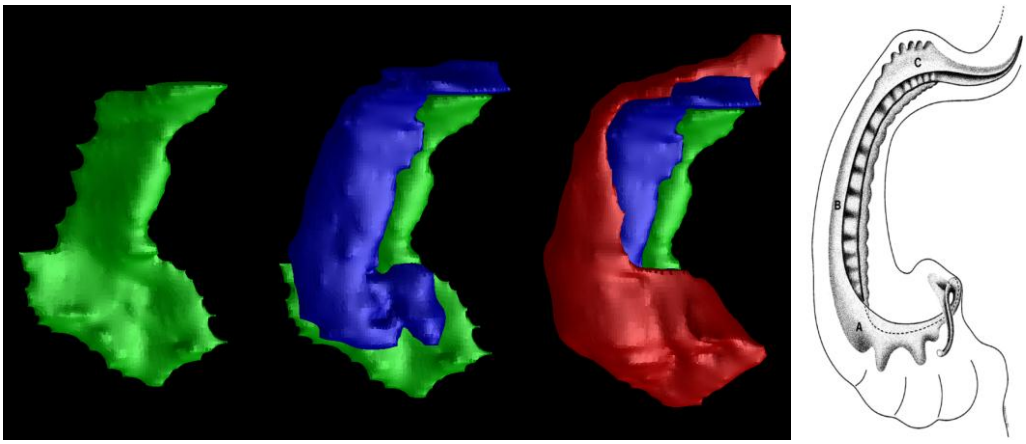


Figure 5.6: A 3-D reconstruction of the right HC (with the anterior end on the bottom), segmented into its subfields, using our protocol, as seen from a superior perspective (top view).
Extreme left: Sub
Centre-left: Sub + DG
Centre-right: Sub + DG + CA
The CA is not shown in the extreme-left and centre-left pictures while the DG is not shown on the extreme-left to give more unobstructed views of the DG and Sub.
The picture on the extreme right shows the anatomical location and shape of the subfields, as drawn from studies on post-mortem hippocampi; A. hippocampal head, B. body, C. tail
(Duvernoy, 2005) (Appendix B)

5.3.3 Reliability Testing

The reliability tests were done by applying our protocol to a sample of 6 brains (12 hippocampi) compared individually, with intra-rater reliability calculated using segmentations done twice by the same individual and inter-rater reliability calculated using segmentations done by two individuals. Volumes for each hippocampal subregion (hippocampal tail, body, and head) and subfield (CA, DG, Sub), along with total hippocampal volumes, were taken for each of the twelve hippocampi analyzed for this reliability test to obtain intraclass correlation coefficient (ICC) and error as a coefficient of variation (CV) values of the log-transformed data for both intra- and inter- rater reliabilities (see Table 5.2).

Following the protocol yielded good intra- and inter-rater reliability results, showing consistency for each tracer and between different tracers, respectively. This consistency shows the tracers' reliable segmentation of the HC along its longitudinal axis into the hippocampal head, body, and tail, and also into its subfields – the CA, DG, and Sub, as the R-values for each of these sections is above 0.80. Raw volumes of the HC were used to assess this reliability.

Intra-rater reliability							
	Tail	Body	Head	CA	DG	Sub	HC
Intraclass R	0.84	0.90	0.98	0.92	0.81	0.95	0.98
Error as a CV (%)	7.25	4.08	2.42	3.92	5.97	2.95	1.70
Inter-rater reliability							
	Tail	Body	Head	CA	DG	Sub	HC
Intraclass R	0.83	0.82	0.88	0.92	0.81	0.87	0.95
Error as a CV (%)	6.83	6.24	6.59	4.56	6.07	6.50	2.78

Table 5.2: Reliability for tracing the HC

As ICVs were used to normalize all hippocampal volumes, reliable ICV tracing also had to be ensured. The reliability tests for ICV measurements were done in the same way as for the hippocampal regions but using the ICVs of 10 healthy controls for the intra-rater and 10 patients for inter-rater measurements instead, by following the protocols for ICV measurements. Both intra-rater and inter-rater reliabilities for ICV volumetrics, 0.996 and 0.999, were excellent, showing that this variable can also be very reliably measured.

5.4 Statistical Analyses

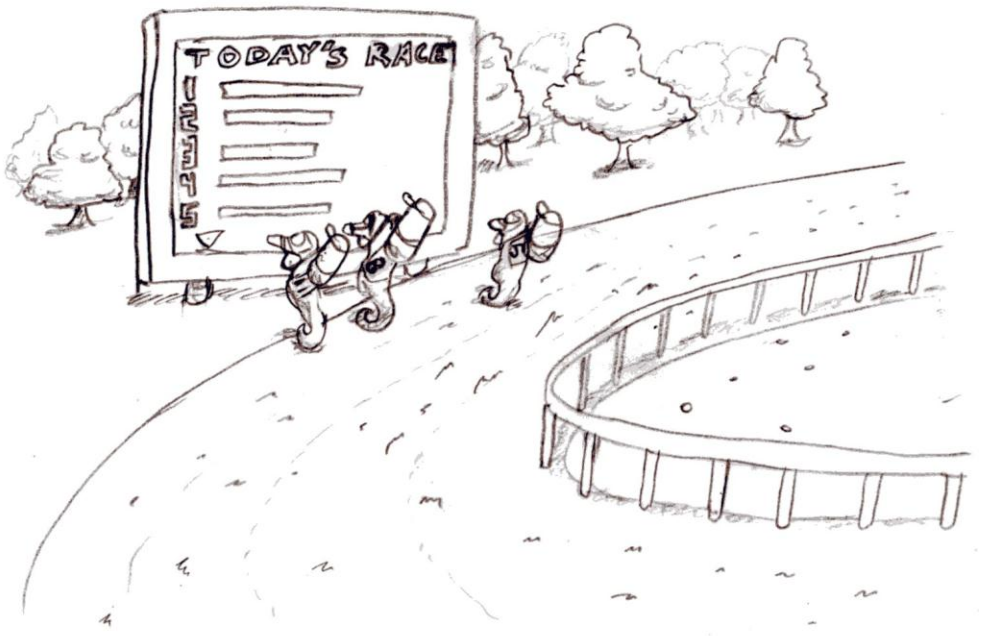
The demographic variables (age, sex ratio, education) along with ICV values were all tested for significance (see Table 6.1 in the Results section) to ensure that representative groups did not differ from each other in factors other than depression and antidepressant treatment that could contribute to different volumes in hippocampal subfields to potentially skew any part of the sample.

Volumes of all three subfield areas (CA, DG, and Sub) in all three subregions (hippocampal tail, body, and head) were statistically evaluated between groups, for a total of 9 sections for comparison per HC. Statistics were done using the software package Predictive Analytics Software (PASW), originally known as the Statistical Package for the Social Sciences (SPSS). Groups were compared (with the volume in question as a dependent variable and hemisphere as a within-subject factor) using one-way analysis of covariance (ANCOVA) with ICV as a covariate to control for varying brain sizes. ANCOVA was done on both a two-group analysis (where MDD patients, both unmedicated

and medicated, were combined into one group and compared with the healthy controls – forming the binary variable of "diagnosis", which was used as a fixed factor in the ANCOVA) and a three-group analysis (where the medicated and unmedicated subjects were separated and compared with the healthy controls as three distinct groups – forming the ternary variable of "treatment", which was used as a fixed factor in the ANCOVA). Diagnosis × hemisphere and group × hemisphere interactions for two-group and three-group analyses, respectively, were observed for any asymmetrical effects of depression on the two different hippocampi. The threshold for significance for the ANCOVA was set to $p < 0.05$. Values for the F-distribution were also calculated for each analysis' respective degrees of freedom (d.f.). Corrections for multiple comparisons was not necessary because our experiment involved an *a priori* hypothesis in respect to specific changes in hippocampal subfields.

Chapter 6

Results



6.1 Demographic Statistics

Both unmedicated (n = 9) and medicated (n = 11) MDD groups, along with healthy controls (total n = 27) (see Tables 6.1 and 6.2) were all right handed and prospectively matched for demographic variables (age, sex, education) along with ICV. MDD patients did not differ in age, sex, education, and ICV from healthy subjects and between subgroups (medicated versus unmedicated). Diagnosis (MDD versus healthy controls) × hemisphere and treatment (unmedicated MDD versus medicated MDD versus or healthy controls) × hemisphere interactions were not significant for any hippocampal subregions or subfields (all $p > 0.05$) (see Tables 6.3 and 6.4). The absence of significant inter-hemispheric interactions suggests that all changes were present bilaterally.

	Healthy Controls	Unmedicated MDD	Medicated MDD	Total MDD
Subjects (**n**):	27	9	11	20
Age (years):				
Mean ± SD	32.8 ± 9.9	32.7 ± 11.6	36.9 ± 9.6	35.0 ± 10.5
Male/female, n	8 /19	5/4	5/6	10/10
Female, %	70.4	44.4	54.5	50.0
Education:				
12 years:	1 (3.7%)	1 (11.1%)	0 (0.0%)	1 (5.0%)
13-15 years:	8 (29.6%)	2 (22.2%)	3 (27.3%)	5 (25.0%)
16 or more:	18 (66.7%)	6 (66.7%)	8 (72.7%)	14 (70.0%)
ICV (cm^3)**:**				
Mean ± S.D.	1576 ± 163	1662 ± 155	1583 ± 89	1618 ± 126

Table 6.1: Subject characteristics. The "Total MDD" column is a group combining the unmedicated with medicated MDD patients together.

	Unmedicated MDD vs. controls	Medicated MDD vs. controls	Unmedicated MDD vs. medicated	Total MDD vs. controls
Age:	p = .978	p = .263	p = .359	p = .462
Sex:	p = .176	p = .372	p = .649	p = .162
Education:	p = .741	p = .639	p = .512	p = .905
ICV:	p = .141	p = .903	p = .241	p = .343

Table 6.2: P-values of demographic variables and ICV

6.2 Hippocampal Subregional Volumes

ANCOVA revealed that MDD patients combined did not differ in total hippocampal subregion volumes from healthy controls (see Table 6.3). In spite of this lack of differences, after separating MDD patients into unmedicated and medicated groups, the medicated MDD patients had larger ($p = 0.02$) total hippocampal tail volumes than the unmedicated MDD patients (see Figure 6.1A and Table 6.4). The unmedicated MDD patients had smaller ($p = 0.03$) total hippocampal body volumes than healthy controls (see Figure 6.1B). In addition to the unmedicated MDD patients' smaller hippocampal tail volumes than medicated MDD patients, a trend was also observed toward smaller hippocampal tail volumes in the unmedicated MDD patients than healthy controls ($p = 0.06$). Furthermore, trends were also observed for smaller total volumes of the HC in unmedicated MDD patients compared to healthy controls and medicated MDD patients ($p = 0.10$ and $p = 0.08$, respectively) (see Figure 6.1D). In addition, a weak trend was observed for the medicated MDD patients having a larger hippocampal body volume than the unmedicated patients ($p = 0.12$). Medicated MDD patients did not differ from healthy controls for any total subregional volumes, and we did also not find significant differences in total hippocampal head volume between any of the groups we studied (see Figure 6.1C).

	Controls	MDD (combined)	Diagnosis[a]		Treatment[b]	
			$F_{(d.f.=1)}$	p	$F_{(d.f.=2)}$	p
ICV	1576 ± 163	1618 ± 126	0.91	.34	1.16	.32
ANCOVA (covariate: ICV)						
Total subregion						
HC tail	579 ± 141	568 ± 157	0.23	.63	**3.33**	**.04***
	(Diag./or/tx.)×	hemisphere	0.02	.86	0.11	.89
HC body	1263 ± 176	1231 ± 196	0.85	.35	**3.06**	**.05***
	(Diag./or/ tx.)×	hemisphere	0.43	.50	0.54	.58
HC Head	1848 ± 322	1923 ± 372	0.07	.78	0.13	.87
	(Diag./or/ tx.)×	hemisphere	0.02	.87	0.12	.87
Total HC	3690 ± 396	3722 ± 503	0.17	.67	**2.84**	**.06**
	(Diag./or/ tx.)×	hemisphere	0.07	.79	0.43	.65
CA						
HC tail	221 ± 68	231 ± 65	0.37	.54	1.75	.17
	(Diag./or/ tx.)×	hemisphere	0.11	.73	0.23	.79
HC body	346 ± 73	311 ± 61	**5.60**	**.02***	**3.45**	**.03***
	(Diag./or/ tx.)×	hemisphere	0.11	.74	0.44	.64
HC head	1051 ± 81	1105 ± 238	0.04	.82	0.06	.93
	(Diag./or/ tx.)×	hemisphere	0.14	.70	0.29	.74
Total CA	1618 ± 282	1646 ± 277	0.11	.74	0.21	.80
	(Diag./or/ tx.)×	hemisphere	0.10	.75	0.53	.58
DG						
HC tail	312 ± 100	289 ± 107	1.56	.21	**3.06**	**.05***
	(Diag./or/ tx.)×	hemisphere	0.00	.99	0.04	.95
HC body	571 ± 105	564 ± 111	0.25	.61	2.03	.13
	(Diag./or/ tx.)×	hemisphere	0.28	.59	0.38	.67
HC head	399 ± 63	409 ± 99	0.10	.75	**2.67**	**.07**
	(Diag./or/ tx.)×	hemisphere	0.23	.63	0.17	.84
Total DG	1281 ± 179	1261 ± 244	0.56	.45	**4.82**	**.01***
	(Diag./or/ tx.)×	hemisphere	0.00	.92	0.08	.92
Sub						
HC tail	46 ± 15	49 ± 16	1.13	.28	**3.25**	**.04***
	(Diag./or/ tx.)×	hemisphere	0.02	.88	0.07	.93
HC body	345 ± 74	356 ± 79	0.42	.51	1.69	.19
	(Diag./or/ tx.)×	hemisphere	1.39	.24	0.70	.49
HC head	398 ± 72	410 ± 116	0.00	.93	0.00	.99
	(Diag./or/ tx.)×	hemisphere	0.95	.33	0.50	.60
Total Sub	790 ± 108	815 ± 135	0.35	.55	1.10	.33
	(Diag./or/ tx.)×	hemisphere	0.00	.93	0.02	.97

Table 6.3: Comparison of volumes between the controls and MDD groups. Volumes for ICV, the HC, its subfields and subregions are in mm^3. Significant values (*$p < .05$) and trends ($p \leq 0.10$) and are in **bold**.

[a] Diagnosis (Diag.) compares controls vs. MDD.

[b] Treatment (tx.) compared the controls vs. Unmedicated MDD vs. Medicated MDD

	Unmedicated MDD	Medicated MDD	Unmedicated MDD vs. controls		Medicated MDD vs. controls		Medicated vs. unmedicated MDD	
			$F_{(d.f.=1)}$	p	$F_{(d.f.=1)}$	p	$F_{(d.f.=1)}$	p
ICV	1662 ± 155	1583 ± 89	1.89	.14	0.01	.90	2.03	.24
ANCOVA (covariate: ICV)								
Total Subregion								
Total HC tail	511 ± 145	615 ± 153	**3.62**	**.06**	0.99	.32	**5.44**	**.02***
	Group ×	hemisphere	0.02	.88	0.15	.69	0.18	.67
Total HC body	1163 ± 190	1287 ± 188	**4.78**	**.03***	0.23	.63	2.42	.12
	Group ×	hemisphere	0.00	.99	1.08	.30	0.62	.43
Total HC head	1955 ± 473	1896 ± 272	0.00	.99	0.29	.58	0.50	.48
	Group ×	hemisphere	0.16	.68	0.02	.87	0.18	.66
Total HC	3629 ± 629	3798 ± 369	**2.74**	**.10**	1.44	.23	**3.13**	**.08**
	Group ×	hemisphere	0.11	.73	0.86	.35	0.50	.48
CA								
HC tail	212 ± 50	246 ± 72	0.30	.58	2.04	.15	**3.48**	**.07**
	Group ×	hemisphere	0.01	.91	0.34	.55	0.38	.54
HC body	296 ± 60	323 ± 61	**5.41**	**.02***	1.74	.19	1.39	.24
	Group ×	hemisphere	0.64	.42	0.04	.83	0.98	.32
HC head	1156 ± 304	1063 ± 163	0.13	.72	0.00	.97	0.00	.99
	Group ×	hemisphere	0.01	.89	5.55	.46	0.53	.46
Total CA	1664 ± 337	1632 ± 223	0.22	.63	0.00	.96	0.61	.44
	Group ×	hemisphere	0.13	.71	0.77	.38	0.96	.33
DG								
HC tail	257 ± 97	314 ± 110	**5.28**	**.02***	0.01	.91	**3.61**	**.06**
	Group ×	hemisphere	0.03	.84	0.02	.86	0.08	.77
HC body	532 ± 92	589 ± 120	**2.87**	**.09**	0.41	.52	1.69	.20
	Group ×	hemisphere	0.00	.97	0.69	.40	0.47	.49
HC head	380 ± 94	432 ± 98	1.61	.20	**2.68**	**.10**	**3.48**	**.07**
	Group ×	hemisphere	0.03	.85	0.39	.53	0.06	.79
Total DG	1169 ± 224	1336 ± 241	**6.82**	**.01***	1.13	.29	**4.79**	**.03***
	Group ×	hemisphere	0.03	.84	0.09	.75	0.10	.74
Sub								
HC tail	42 ± 14	54 ± 16	0.37	.54	**4.57**	**.03***	**4.93**	**.03***
	Group ×	hemisphere	0.12	.72	0.00	.94	0.11	.73
HC body	335 ± 70	374 ± 82	0.64	.42	2.13	.14	0.95	.33
	Group ×	hemisphere	0.74	.39	1.02	.31	0.01	.91
HC head	420 ± 135	402 ± 100	0.01	.91	0.00	.92	0.13	.71
	Group ×	hemisphere	0.88	.35	0.52	.47	0.05	.82
Total Sub	796 ± 156	830 ± 118	0.24	.62	1.87	.17	1.00	.32
	Group ×	hemisphere	0.00	.93	0.04	.83	0.03	.85

Table 6.4: Comparison of volumes between the controls,unmedicated MDD, and medicated MDD groups. Volumes for ICV, the HC, its subfields and subregions are in mm^3.Significant values (* $p < .05$) and trends ($p \leq 0.10$) and are in **bold**.

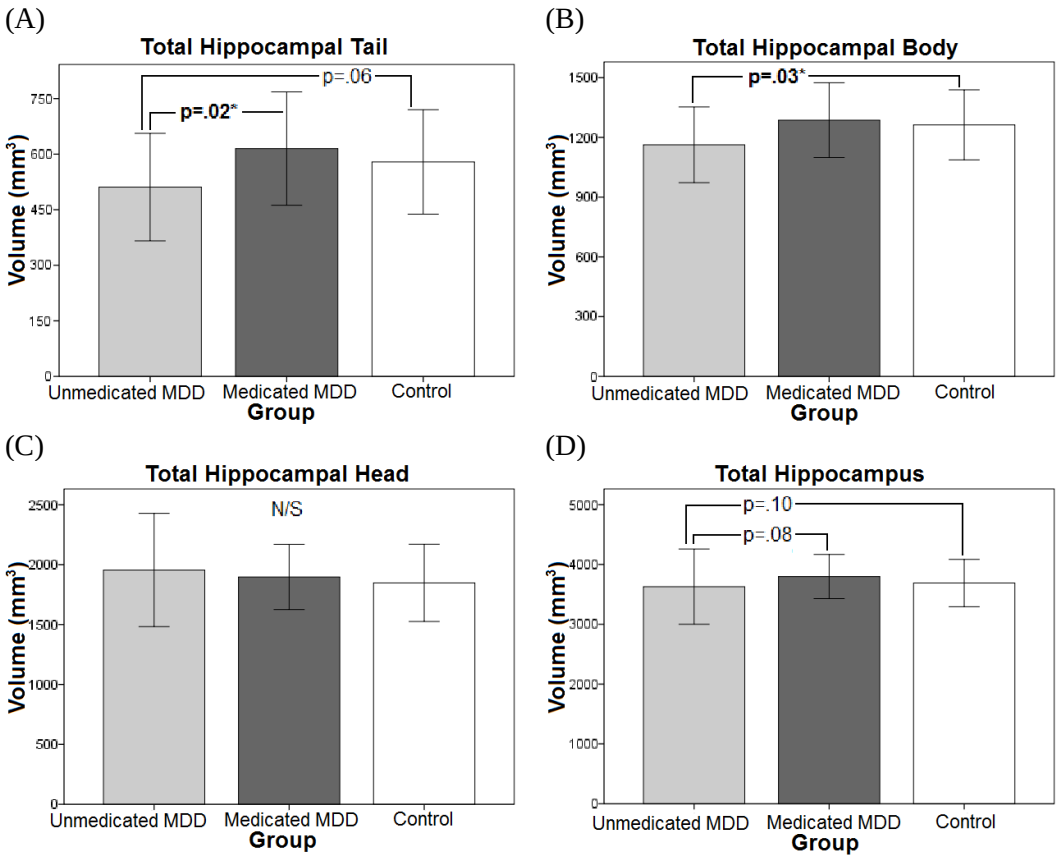


Figure 6.1: Total mean volumes for the hippocampal subregions:
(A) the hippocampal tail, (B) body, and (C) head, along with (D) total hippocampal volume. P-values of $p \leq .10$ from an ANCOVA of the groups are shown. Error bars display standard deviation (SD) from the mean.

6.3 Hippocampal Subfield Volumes

MDD patients (as a combined group) had significantly smaller ($p = 0.02$) CA volumes in the hippocampal body, although no other differences were observed for other hippocampal subfields between these groups (see Table 6.3). ANCOVA revealed additional associations of treatment with hippocampal subfield volumes. The unmedicated MDD patients had significantly smaller ($p = 0.02$) hippocampal body CA volumes than the healthy controls (see Figure 6.2B and Table 6.4). This difference was more prominent than the weak trend seen in the CA of the hippocampal body of medicated patients being smaller than the healthy controls' ($p = 0.19$), while unmedicated and medicated MDD patients did not differ in this region (see Figure 6.2B and Table 6.4). Medicated MDD patients had a trend toward larger CA volumes in the hippocampal tail than unmedicated patients ($p = 0.07$), but unmedicated MDD patients did not show any differences from the healthy controls for this area (see Figure 6.2A). The CA volumes in the hippocampal head and total CA did not differ between the groups (see Figures 6.2C and 6.2D, respectively).

ANCOVA did not reveal any effect of diagnosis on DG volumes, either for the total DG or within hippocampal subregions (see Table 6.3). Notwithstanding, when the MDD patients were separated based on treatment, unmedicated MDD patients showed significantly smaller volumes of the total DG than both the healthy controls ($p = 0.01$) and the medicated MDD patients ($p = 0.03$) (see Figure 6.3D and Table 6.4). Smaller volumes of the DG in unmedicated MDD patients were observed in the hippocampal tail ($p = 0.02$), with trends

toward smaller volumes in the hippocampal body ($p = 0.09$) and head ($p = 0.20$) (see Table 6.4). In addition, medicated MDD patients had trends toward larger DG volumes than unmedicated MDD patients in all hippocampal subregions: the hippocampal tail ($p = 0.06$), body ($p = 0.20$), and head ($p = 0.07$) (see Table 6.4).

Although MDD patients altogether did not differ from healthy controls for volumes of any areas in the Sub following an ANCOVA (see Table 6.3), we found that medicated MDD patients had a larger volume of the Sub in the hippocampal tail than either the unmedicated MDD patients ($p = 0.03$) or controls ($p = 0.03$) (see Figure 6.4A and Table 6.4). Further analysis did not reveal any other effects of diagnosis or treatment on the volumes of Sub (see Figures 6.4B-D).

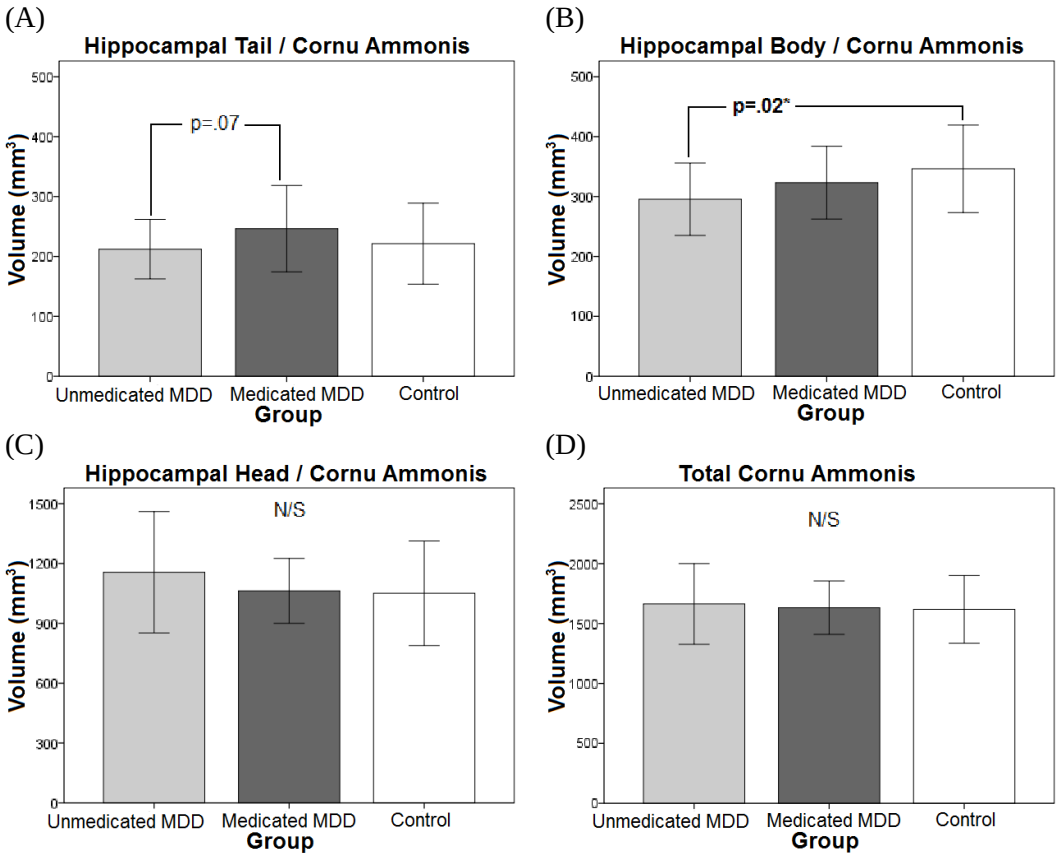


Figure 6.2: Mean volumes for the CA in hippocampal subregions:
(A) the hippocampal tail, (B) body, (C) head, and (D) total CA volume. P-values of $p \leq .10$ from an ANCOVA of the groups are shown. Error bars display SD from the mean.

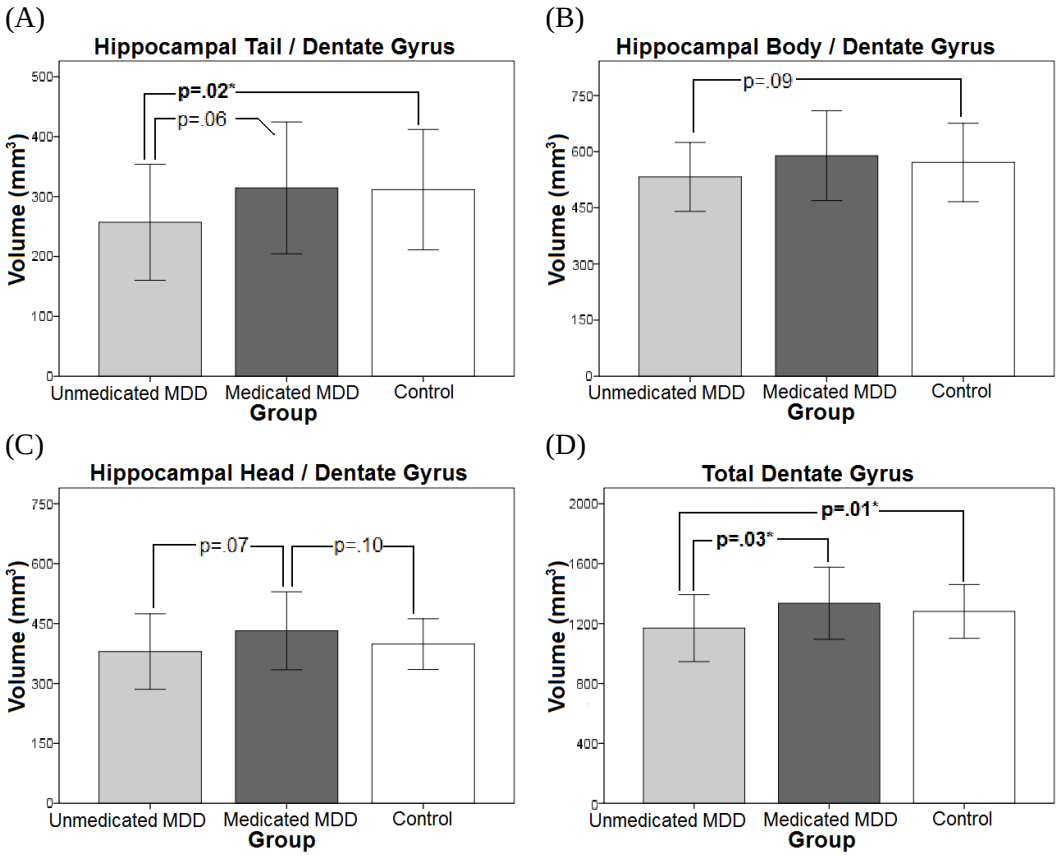


Figure 6.3: Mean volumes for the DG in the various subregions:
(A) the hippocampal tail, (B) body, (C) head, and (D) total DG volume. P-values of $p \leq .10$ from an ANCOVA of the groups are shown. Error bars display SD from the mean.

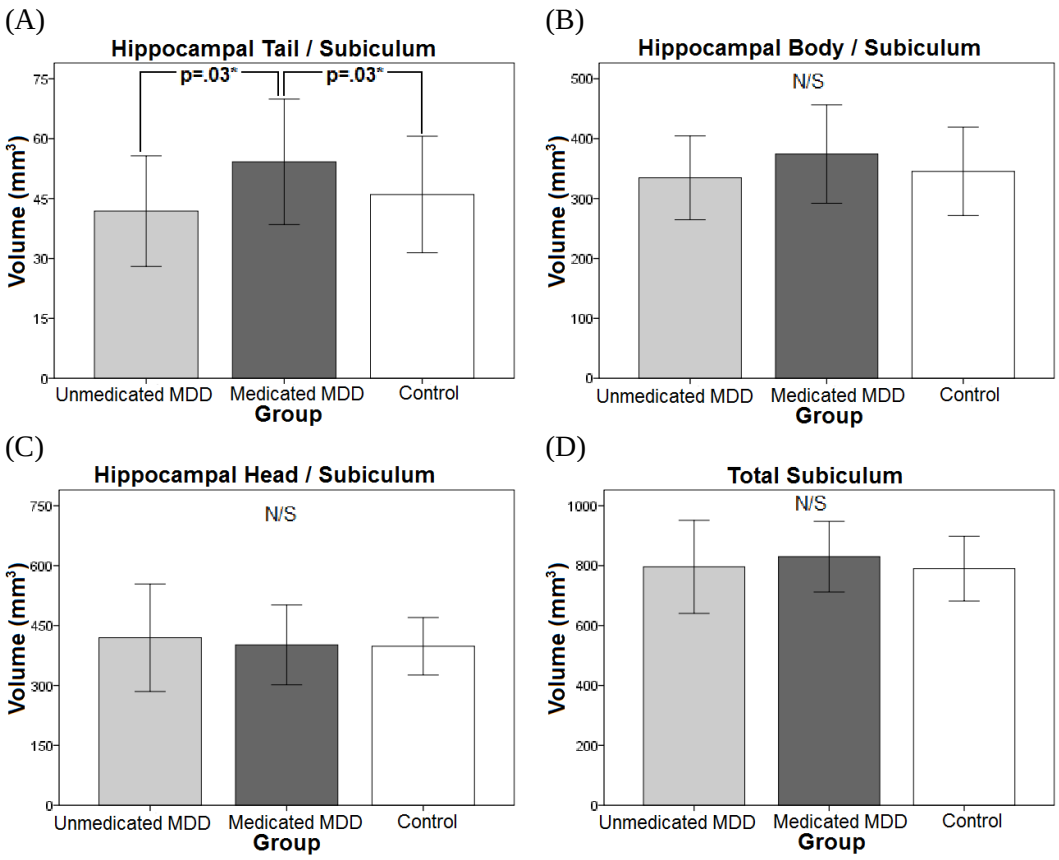
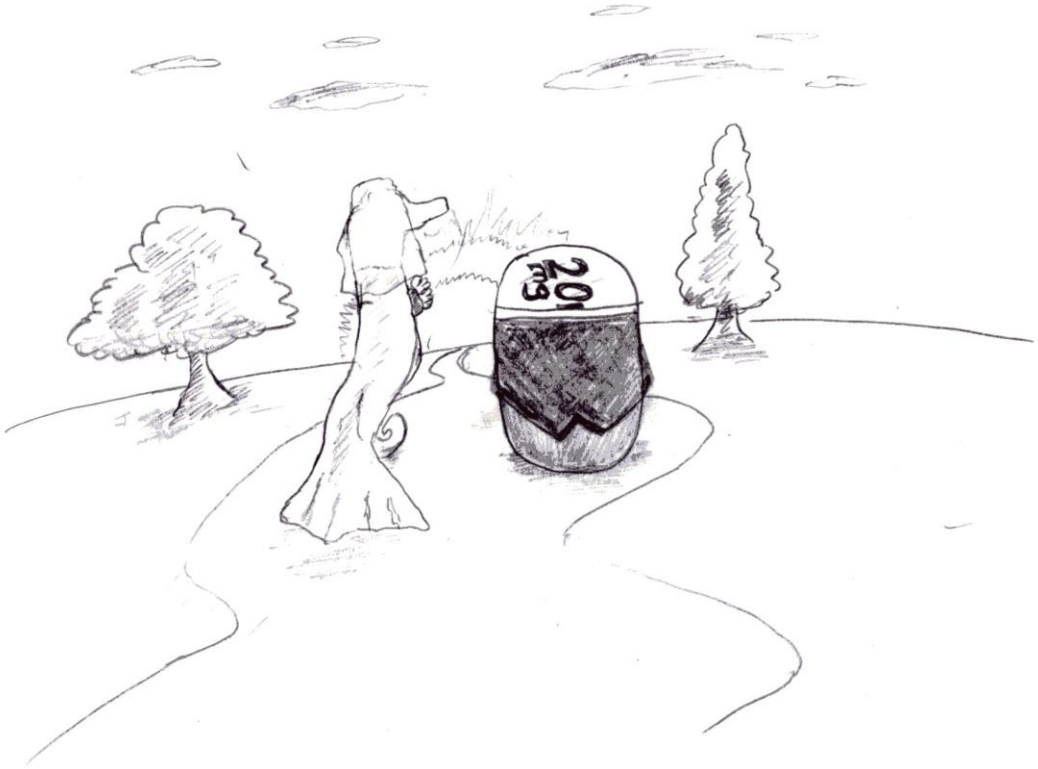


Figure 6.4: Mean volumes for the Sub in the various subregions:
(A) the hippocampal tail, (B) body, (C) head, and (D) total Sub volume. P-values of $p \leq .10$ from an ANCOVA of the groups are shown. Error bars display SD from the mean.

Chapter 7

Discussion



7.1 General Findings

To our knowledge, this was the first high field volumetric MRI study to examine hippocampal subfields *in vivo* in patients with MDD. Participants with MDD had smaller CA subfield volumes in the hippocampal body bilaterally compared with controls. Furthermore, unmedicated MDD patients had lower total DG volumes bilaterally compared to either medicated MDD patients or healthy controls. The smaller DG volumes in unmedicated patients were found in the total DG and hippocampal tail, with trends in the DG in the other subregions. We found that in unmedicated MD patients the smaller CA and DG subfields of the HC, but not in the Sub, contributed to overall smaller volume of the HC and its tail and body subregions compared to healthy controls and medicated MDD patients. Our results are in agreement with the preclinical studies of chronic stress in adult animals (Joels et al., 2004; Leuner & Gould, 2010; McEwen, 2001; Sapolsky, 2000) that showed significant decreases in CA3 volume, massive loss due to neuronal death of CA3 pyramidal cells in the HC of severely stressed animals, dendritic retraction in the CA (CA1-3) and DG, and suppressed DG neurogenesis and glial loss, all apparently involving elevated levels of glucocorticoids, and therefore suggested as possible causative factors in HC shrinkage (Czeh & Lucassen, 2007). In addition, our results suggest that antidepressant treatment can increase volumes of hippocampal subfields and subregions, which have been observed to be smaller in unmedicated MDD patients.

7.2 Differences in the Global Volume of the Hippocampus and its Subregions Associated with MDD and Antidepressant Treatment

Numerous, but not all, previous volumetric MRI studies have reported hippocampal volume reductions in MDD (McKinnon et al., 2009; Videbech & Ravnkilde 2004). More recent studies (MacQueen et al., 2008; Maller et al., 2007; 2012; Malykhin et al., 2010b) investigated changes in hippocampal subregions and reported that those subregions were not uniformly affected in MDD patients. For instance, several studies reported reductions in the volume of the hippocampal tail (Maller et al., 2007; 2012; Malykhin et al., 2010b; Neumeister et al., 2005). In addition, posterior (body + tail) hippocampal volumes increases were reported in every single MDD patient after antidepressant treatment (Schermuly et al., 2011) while remitted MDD patients had larger pretreatment hippocampal body-tail volumes at baseline (MacQueen et al., 2008). Additionally, in our recent cross-sectional study in MDD patients, we demonstrated for the first time that individuals on long term antidepressant treatment (median 36 months) had larger hippocampal body volumes than patients off antidepressants (median 12 months) (Malykhin et al., 2010b). These findings suggested differential vulnerability of hippocampal subregions. The results of the present study are in agreement with the previous studies (Maller et al., 2007; 2012; Malykhin et al., 2010b; Neumeister et al., 2005) suggesting that the hippocampal tail might be particularly vulnerable in MDD patients. Also in agreement with our previous study, we found

that only unmedicated MDD patients tended to have smaller total volumes of the HC compared to controls, while medicated MDD subjects and controls did not differ (Malykhin et al., 2010b). In contrast to our previous study (Malykhin et al., 2010b), in the present study we also found that unmedicated MDD patients had smaller volumes of the hippocampal body compared to the healthy subjects. There are two possible factors in the methodology that could account for this discrepancy. First, half of the unmedicated patients in the present study were medication naïve compared to our previous study where the majority of unmedicated MDD patients were only medication free but not naïve. Second, in our previous volumetric study, the white matter of the fimbria was included in the volume of hippocampal body, since the resolution simply did not allow us to separate it reliably from grey matter of the HC, whereas this volumetric study's images had sufficient resolution to separate it. However, in agreement with our previous study, antidepressant treatment had a significant effect on volume of the hippocampal body. In addition, also in agreement with our previous study, medicated patients showed larger volumes of hippocampal tail and tended to have larger global hippocampal volumes compared to unmedicated patients. Overall, our data suggest that antidepressant treatment was associated with increases in volumes of the hippocampal tail, hippocampal body and global volume of the HC, but not the hippocampal head as we have also previously demonstrated (Malykhin et al., 2010b).

Despite the fact that the reduction of hippocampal volume in MDD has been confirmed in many MRI volumetric studies, there were too few longitudinal

studies to determine if hippocampal volume loss is progressive or influenced by antidepressants (Frodl et al., 2008a; 2008b; Schermuly et al., 2011; Vythilingam et al., 2004). Administration of antidepressants for less than one year did not increase the volume of the HC in MDD (Vythilingam et al., 2004), but did so in PTSD (Vermetten et al., 2003). Although a robust 3 year prospective study yielded negative results on hippocampal changes between depressed participants and healthy controls (Frodl et al., 2008a; 2008b), our results are still not conflicted because the results may have differed due to either the use of different methods of hippocampal tracing or the use of VBM by the 3 year prospective study. Tracing showed increased left hippocampal volumes with medication that were independent of clinical outcome, whereas VBM showed volume losses in MDD patients with a poor outcome, but no effect of antidepressant treatments. These discrepancies might be because VBM is sensitive to local rather than total volume differences: it does not co-register hippocampal boundaries (Amunts et al., 2005), possibly due to the HC being a structure that is highly polymorphic amongst the population with its highly variable boundaries (Pruessner et al., 2000).

Another longitudinal study that reported posterior hippocampal volume increases (Schermuly et al., 2011) also suggested that hippocampal growth occurs independently from behavioural and cognitive changes (e.g. depression severity or memory performance), confirming the evidence from animal studies that the specific suppression of hippocampal neurogenesis does not in itself produce depression-like behaviour in animals (Airan et al., 2007; Santarelli et al., 2003;

Saxe et al., 2006; Shors et al., 2002), even though the recovery of neurogenesis still is likely required for the recovery from stress (Kempermann & Kronenberg, 2003; Santarelli et al., 2003).

The results of the present cross-sectional study provide evidence of possible neuroprotective effects of antidepressant treatment in MDD patients and could also explain the discrepancies between some previous volumetric MRI studies in MDD that did not find significant differences between controls and MDD patients (Caetano et al., 2004; Posener et al., 2003; Vakili et al., 2000; von Gunten et al., 2000), the majority of whom were on antidepressant treatment.

7.3 Differences Amongst Hippocampal Subfields in MDD

MDD patients showed significantly smaller volumes than the healthy controls' bilaterally in their CA volumes within the hippocampal body. However, this difference was only in unmedicated patients ($p = 0.02$) and was only a weak trend in medicated patients ($p = 0.19$) compared to healthy controls. Although the CA subfield volumes within the hippocampal tail did not differ between MDD patients and controls, unmedicated MDD patients tended to have smaller volumes compared to medicated MDD ($p = 0.07$).

Antidepressant treatment showed a significant effect on the volume of the CA subfield in the hippocampal body but only a trend ($p = 0.17$) on the CA volume in the hippocampal tail. We found that overall, MDD patients did not differ from controls in either the total volumes of the DG or the DG volumes

within hippocampal subregions. However, in unmedicated patients, lower DG volumes or trends toward lower volumes were found in all hippocampal subregions along with lower global DG volumes, in comparison to healthy subjects. Furthermore, antidepressant treatment was associated with significantly larger total volumes and trends for larger subregional volumes of the DG within the HC.

These observations on the changes in the DG of the HC are in line with the neurogenic hypothesis of depression suggesting that the reduction in the rate of neurogenesis during depression (i.e. a reduced production of new neurons) in the DG plays a role in low hippocampal volumes in MDD, and that successful antidepressant treatment requires an enhancement in hippocampal neurogenesis, which may involve the recovery of the DG volumes (Jacobs et al., 2000; Kempermann & Kronenberg, 2003). Preclinical studies have demonstrated that both acute and chronic stress paradigms and various animal models of MDD involve the suppression of adult HC neurogenesis (Czeh et al., 2001; 2002; Pham et al., 2003). However, our volumetric findings on the hippocampal subfields are limited since we were not able to evaluate the rate of neurogenesis within the DG directly or, as with post-mortem studies, use NPCs to estimate the level of production of new neurons in the HC (Boldrini et al., 2009). Therefore, our finding on the volumetric changes in the DG only indirectly provide evidence of hippocampal neurogenesis, suggesting that the relative size of the DG is related to the extent of this neurogenesis.

Our results suggest that antidepressant treatment in fact might have a direct *in vivo* effect on the neuroplasticity of the DG and CA, as has been suggested previously in preclinical studies (Czeh & Lucassen, 2007). Although the selective disruption of DG neurogenesis does not induce any depressive-like behaviours directly, it can block the behavioural effects of antidepressants (Santarelli et al., 2003). Chronic, but not acute, administration of several types of antidepressants can not only reverse the stress-induced suppression of neurogenesis, but also increase basal DG neurogenesis in the absence of experimental stressors (Czeh et al., 2001; Dranovsky & Hen, 2006; Malberg et al., 2000; Santarelli et al., 2003).

The results of the present study suggest that antidepressant treatments were associated with the increased volumes of all subfields and subregions that were found to be smaller in unmedicated MDD patients. Interestingly, the volume increases did not depend on sustained remission, which suggests that they could be a direct effect of the antidepressant treatment rather than the result of successful remission.

The only hippocampal subfield that did not differ between MDD patients and controls was the Sub. This finding is line with our initial hypotheses and the post-mortem and animal studies that showed that the CA and DG were more vulnerable to stress and depression. However, effects of antidepressants on the Sub subfield are understudied. In our study, antidepressant treatment was associated with a higher volume of Sub in the hippocampal tail compared to either the unmedicated or control groups. This finding might be explained by several

factors. First, the only hippocampal subregion that was overall significantly smaller between unmedicated and medicated MDD patients was the hippocampal tail and its larger volume was associated with antidepressant treatment, which could be explained by the assumption that changes in neuroplasticity within the hippocampal tail associated with depression and treatment are more prominent than in other hippocampal subregions. Second, medicated MDD patients tended to have trends toward larger total volumes of Sub ($p = 0.17$) and also larger volumes for the subfield within the body ($p = 0.14$) and the tail ($p = 0.03$) that overall mirror the effects of treatment on global hippocampal volume and volume of the hippocampal tail and body. Although these findings did not reach significance at $p < 0.05$ except for at the tail, they could be an indication that the effects of antidepressant treatment are not limited to previously reduced hippocampal subfields (CA and DG), but might also involve neuroplastic changes in the Sub. Several preclinical studies have also confirmed that the Sub is likely to be the primary area of hippocampal interactions with the HPA axis (Herman & Mueller, 2006; O'Mara, 2006). The ventral Sub has also been shown to be critical for stress responsiveness (i.e. the inhibition of the HPA axis via negative feedback to heightened glucocorticoid release after psychogenic stress), whereas the dorsal component may gate information concerning basal secretory patterns and is also involved in the integration of information from body movements and memory (Herman & Mueller, 2006; O'Mara, 2006). Recent data from animal studies suggest that diurnal corticosterone rhythms, which are disrupted in depression, are necessary for antidepressants to stimulate neurogenesis (Huang & Herbert, 2006),

which may be relevant to the HPA axis dysregulation in MDD dependent on the ventral Sub. Increased volumes of the dorsal (i.e. posterior in our case) Sub in medicated MDD patients might correspond to a normalization of basal cortisol level; however we did not acquire any cortisol data in our patients in order to be able to answer this question directly.

7.4 Postmortem Studies in MDD and Animal Studies of Stress

Preclinical studies suggest that the CA subfields of the HC, particularly the CA3 pyramidal cells, are most vulnerable to neuronal changes and cell loss associated with prolonged social stress and glucocorticoid overexposure (Joels et al., 2004; Sapolsky, 2000). Additionally, following stress, retraction of dendrites occurs in the CA3 more commonly than in the CA1 or DG, along with the suppression of neurogenesis in the DG (Czeh & Lucassen, 2007). However, glucocorticoid-induced neuronal damage of the HC has not yet been confirmed in human post-mortem brain tissue of severely depressed patients.

A reduction in the rate of neurogenesis in the DG has been suggested to play a role in the low hippocampal volumes observed in MDD (Jacobs et al., 2000). In rodents, proliferative cells migrate from the SGZ into the granular layer, whereas in primates the SGZ is less easily defined and proliferative cells appear in the polymorphic and granular layers and hilus (Boldrini et al., 2009; Eriksson et al., 1998; Lavenex et al., 2009). Adult neurogenesis, a process with a declining

rate throughout an individual's lifetime, has been shown to be suppressed by chronic stress (Balu & Lucki, 2009; Leuner & Gould, 2010). Whereas suppression in healthy adults is transient, early adversity leads to a developmental deficit in adult DG volume and consequently to a lasting suppression of the rate of neurogenesis (Lemaire et al., 2000; Lucassen et al., 2009; Mirescu et al., 2004). However, it is interesting that the specific suppression of hippocampal neurogenesis does not in itself produce depression-like behaviour in animals (Airan et al., 2007; Santarelli et al., 2003; Saxe et al., 2006; Shors et al., 2002). In addition, antidepressants, irrespective of their mechanisms of action, trigger neuronal remodeling and synaptic plasticity and also promote hippocampal neurogenesis but that might not be a critical event for their mood-rectifying actions (Bessa et al., 2009). Chronic stress may also reduce the number, size and processes of HC astrocytes, but this is understudied (Czeh et al., 2006).

In contrast to multiple MRI volumetric studies in MDD, a small number of human post-mortem studies in MDD has not shown sufficient evidence for neuronal death to be confirmed as a significant factor in hippocampal volume reduction in MDD (Lucassen et al., 2001; Muller et al., 2001). They also have limitations of sample power and medication history. In the study of Lucassen et al. (2001) (n = 15), the majority of the MDD patients had been previously treated with antidepressants and the HC proper was dissected at a mid-anteroposterior level (i.e. hippocampal body). The authors suggested that hippocampal apoptosis in MDD was a minor event and was absent from the CA3 region of the hippocampal pyramidal cell layer. However, they found that cells undergoing

apoptotic cell death were localized in other hippocampal subfields such as the CA1, CA4, DG, Sub and entorhinal cortex.

Stockmeier et al. (2004), in their post-mortem MDD study (5 out of 19 MDD patients were unmedicated), examined sections of right HC at the level of the hippocampal body. The authors found a robust and significant difference between control and depressive patients in the thickness of the sections after histological processing. After histological processing, sections from depressive subjects shrank approximately 18% more than sections from control subjects. The authors speculated from this observation that tissue from depressed subjects contained more water (Stockmeier et al., 2004), implying the displacement of functional cellular structure that could have disappeared. Pyramidal neuron density in all CA subfields and granule cell density in the DG were significantly increased by 35% in MDD patients compared to controls. The average soma size of pyramidal neurons in the CA regions was significantly decreased by 17%–21% in MDD patients. In addition, in the granule cell layer of the DG the neuronal soma size was decreased by 22% in MDD. In MDD, the glial cell density was significantly increased by about 30% across the hippocampal pyramidal subfields and granule cell layer of the DG. Neuropil consists of the lattice of glial cells and their processes, dendrites, and proximal axons surrounding neuron cell bodies. The increased cell density in the HC indicates a reduction of neuropil per cell, which may contribute to the volume reduction in the HC in MDD (Stockmeier et al., 2004). Based on the results of their study, it has been hypothesized that a loss in neuropil in processes which include the loss of dendritic branching, dendritic

spine complexity, and glial processes can be related to the decrease in hippocampal volume noted by structural imaging studies in MDD (Stockmeier et al., 2004). However, a recent animal study (Bessa et al., 2009) shows that even with this decrease in neutrophil and volume, antidepressants could still retain their therapeutic efficacy in reducing both measured indices of depression-like behaviour (learned helplessness and anhedonia), even when neurogenesis was blocked. The authors suggested that the reestablishment of other forms of neuronal plasticity (dendritic remodeling and synaptic contacts) in the HC and prefrontal cortex, rather than neurogenesis, was the basis for the restoration of behavioural allostasis by antidepressants.

A recent post-mortem study by Boldrini et al. (2009) revealed that treated MDD patients (n = 7) had larger volumes of DG compared to untreated MDD patients (n = 5) or controls (n = 7). Treated MDD patients also had more NPCs compared to untreated MDD patients and controls. The increase of NPCs and dividing cells in treated MDD patients was localized in the rostral part of the DG. In our study, larger volumes of the DG were observed in medicated MDD patients compared to the unmedicated, while medicated MDD patients did not differ from controls. However, there was a trend for a larger volume in the DG of the hippocampal head in medicated MDD patients ($p = 0.1$) compared to controls which did not reach the 0.05 level of significance. In addition, unmedicated MDD patients showed smaller volumes of the DG compared to both medicated MDD patients and controls, suggesting that an overall reduction in DG volume could be a marker of untreated MDD, while antidepressant treatment might restore these

changes to normal volume similar to healthy subjects. The most intriguing finding of the present study is that antidepressant treatment was associated with larger volumes of both the DG and CA subfields of the HC, suggesting that the neuroplasticity of the HC is not limited to the DG but also occurs in other hippocampal subfields. This is in agreement with previous animal studies that found that antidepressant treatment can affect hippocampal structure by the prevention of CA and DG spine loss (Bessa et al., 2009; Hajszan et al., 2009; Norrholm & Ouimet, 2001), prevention of CA and DG dendritic retraction (Bessa et al., 2009; Magarinos et al., 1999) and prevention of astrocyte loss (Czeh et al., 2006). Our findings on the effects of antidepressants on volume of the CA subfield are also supported by the fact that the CA3 atrophy in rat and tree shrew hippocampi after chronic stress or excess glucocorticoid levels disappeared once the depressive-symptom-inducing treatment was stopped or antidepressant treatment commenced (Margarinos et al., 1996; 1999).

Only two postmortem MDD studies have investigated whether antidepressants affect neurogenesis. One using confirmed treatment with both prescription and toxicological data showed that treatment increased NPCs and that TCA treatment increased proliferative cells (Boldrini et al., 2009). The other did not find an effect, but was significantly weakened by the fact that they only used prescription data to determine diagnosis (Reif et al., 2006).

Since structural changes in individually separated hippocampal subfields have not been previously examined in volumetric MRI studies of MDD due to the limited spatial resolution of conventional MRI and the lack of a comprehensive

analysis of all hippocampal subregions in post-mortem MDD studies (only the hippocampal body was included), it is difficult to predict which different hippocampal subfields within different subregions of the HC are more or less vulnerable in MDD. Our previous study of hippocampal subfields in healthy subjects suggested that the differences in vulnerability of hippocampal parts might be explained by their different structural organization, such as distribution across different subfields (Malykhin et al., 2010a). We reported that the HC head and tail occupy the largest proportion of the CA, and therefore processes that preferentially affect the CA may have a greater impact on the hippocampal head and tail. This suggestion was only partially supported by the results of the present study where the reductions in the CA volumes associated with unmedicated MDD were found in the hippocampal tail and body, but not in the hippocampal head in MDD patients. In addition, since the HC tail has a larger DG/CA ratio than the hippocampal head (Malykhin et al., 2010a), we speculated that it also plays a major role in hippocampal neurogenesis. This hypothesis was supported by the results of the present study, where the hippocampal body and tail were the hippocampal subregions that showed significant association with antidepressant treatment. Furthermore, a trend toward larger volumes in the CA subfield in the hippocampal tail that was associated with treatment (i.e. larger volumes in medicated MDD patients vs. unmedicated patients) was observed only in the hippocampal body, providing more evidence of this hippocampal subfield playing an exceptionally critical role in the antidepressant-induced neuroplasticity. However, larger volumes in the DG of medicated than in unmedicated MDD

subjects were observed in all hippocampal subregions, suggesting that antidepressant-induced neuroplasticity in the DG is not limited to the hippocampal body and is rather manifested throughout the entire hippocampal structure.

Restoration of the volume of the DG in the HC could have a significant effect on the memory function in MDD patients. Although our study investigating how changes in hippocampal subfields are related to memory function in MDD patients is currently ongoing, we have preliminary data supporting this important link (Travis, S. G., Huang, Y., Fujiwara, E., Radomski, A., Carter, R., Seres, P., & Malykhin, N. V., under review). In healthy subjects, the volumes of the DG (especially in the hippocampal tail) were the strongest predictors of memory performance on the standardized tests of the fourth edition of the Wechsler Memory Scale (WMS-IV), a battery of tests on various aspects of memory such as auditory memory, visual memory, and visual working memory, in immediate and delayed settings. In addition, the volume of the CA subfield in the hippocampal body and tail correlated with memory performance in multiple domains. The volume of the hippocampal tail as a whole was strongly associated with memory performance across four out of five memory indices. Of note, global hippocampal volumes, volumes of the Sub, and volumes in the hippocampal head failed to significantly correlate with memory performance in any index of the WMS-IV. Our data suggest that even in healthy young adults, performance in a standard set of neuropsychological memory tests could have a relation to volumes of the DG and CA subfields in the HC. These findings have direct applicability to

the present study: as hippocampal tail volumes are correlated with healthy controls' memory performance and are affected in depression (Maller et al., 2007; Malykhin et al., 2010b); once this study is extended to MDD patients, a potential link might be found in the subregion's volumes, explaining some of the memory deficits seen in depression (Hickie et al., 2005). In the future, we may be able to also see whether the memory deficits in MDD patients are related to specific subfields with the help of these standardized memory scales.

7.5 Limitations

As we were not able to view individual cells and the link between the neurogenesis and antidepressants is not completely clear (neurogenesis not being necessary for all the effects of antidepressants, and compromised neurogenesis not always able to induce the behavioural effects of depression), our study could not conclude that the correlation between antidepressants and the larger volumes is a sure sign of the recovery from depression. In terms of methods, we were limited by the fact that MRIs were susceptible to motion, and our resolution was also limited by the SNR and the time limit of the MRI. Also, our study design was cross-sectional, and prospective studies are needed to determine the direct effects of antidepressants on hippocampal volumes. A relationship between the reported volume differences and memory function or cortisol levels was not determined. Our study sample (n = 20 patients with MDD) was relatively small compared to our previous volumetric study (n = 39) (Malykhin et al., 2010b); however, it was

relatively big compared to the post-mortem studies (n = 12 for Boldrini et al. (2009), n = 15 for Lucassen et al. (2001), and n = 19 for Stockmeier et al. (2004)) reported in the literature, especially since we examined both hippocampi, while Stockmeier et al. (2004) only used the right HC. Our sample size also did not allow examination of sex differences due to small cell sizes. In addition, whether these neuroplastic changes in the HC occur remains to be determined. Previously we showed that long term (> 36 months) antidepressant treatment had an association with increased hippocampal body volume, but it remains unknown if those neuroplastic changes can occur earlier, to a prominent level after simply months or a year of continuous antidepressant treatment. It is still also unclear whether or not the way antidepressant treatments affect hippocampal neuroplasticity are related to the patients' clinical outcome or HPA axis regulation, or are an unrelated side effect. Understanding these mechanisms is very important to the development of novel approaches to MDD.

7.6 Conclusions

Our study showed that lower volumes of the DG and CA subfields of the HC could be state changes of MDD, as they may decrease with depression and recover following antidepressant-assisted remission, or the absence of depression. We also found that long term antidepressant treatment may restore these structural changes in the HC to normal levels in patients with MDD. The higher volumes of the DG could be either trait or state changes depending on whether they remain higher following the cessation of antidepressant administration for a duration of years.

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Appendix A

More Images Illustrating Hippocampal Anatomy

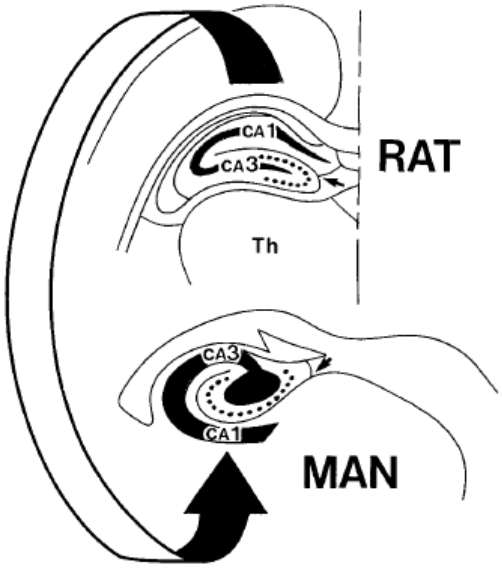

Figure A.1: Differences between the rat (RAT, above) and human (MAN, below) hippocampi
(Duvernoy, 2005) (Appendix B)

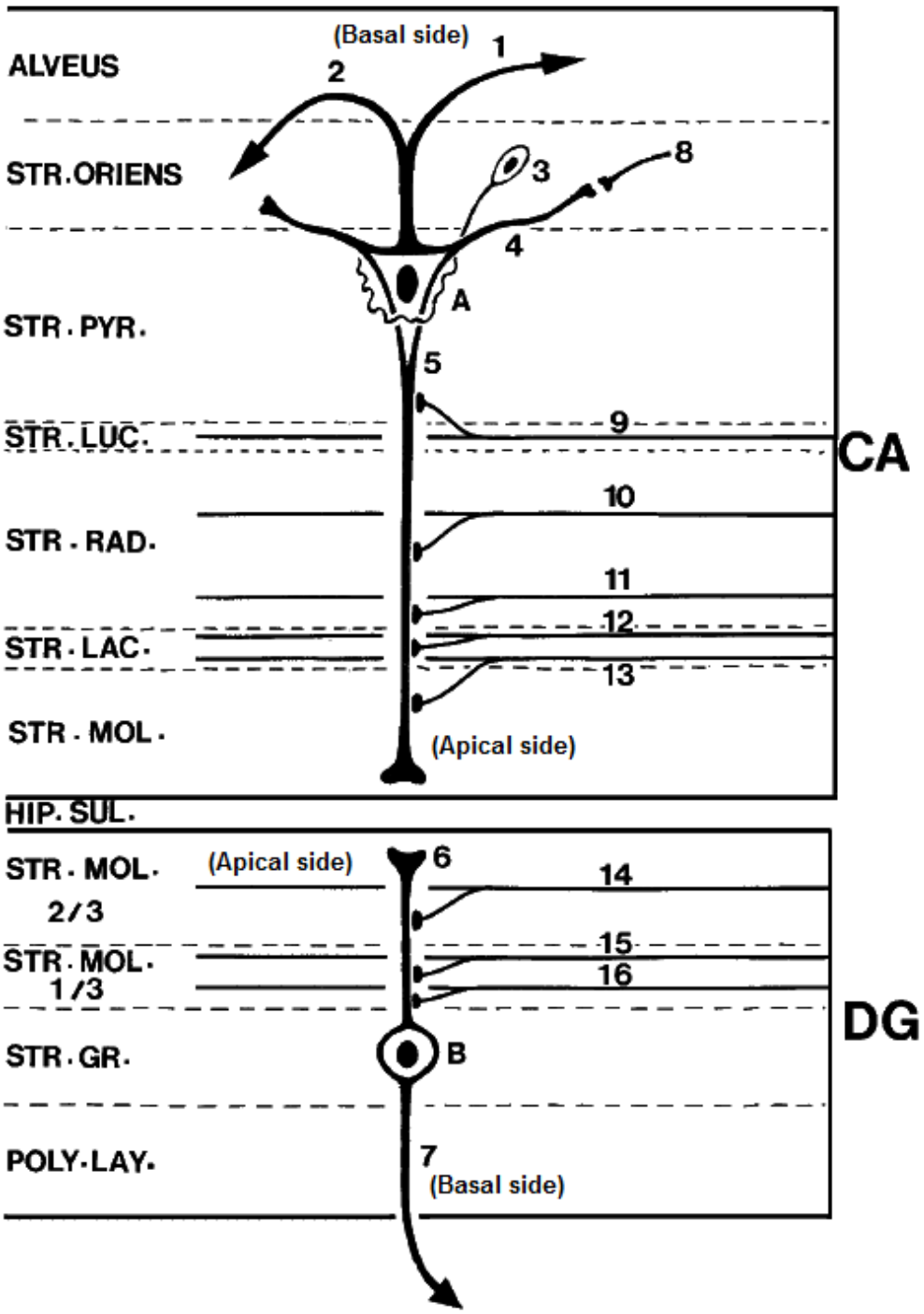

Figure A.2: Layers of the HC; see next page

Figure A.2: Layers of the HC; see previous page

Cornu Ammonis (CA) (top):

A: Pyramidal soma
1. Basal axon
2. Schaffer collateral
3. Basket cell (GABA / inhibitory)
4. Basal dendrite
5. Apical dendrite
8. Connections between basal dendrite of pyramidal neuron and other pyramidal neurons with septal and commissural fibres.
9. Mossy fibres
10. Septal and commissural fibres
11,12. Schaffer collaterals (of other neurons)
13 Perforant path (from entorhinal cortex)

Layers of the CA:
ALVEUS
STR. ORIENS– Stratum oriens
STR. PYR. – Stratum pyramidale
STR. LUC. – Stratum lucidum
STR. RAD. – Stratum radiatum
STR. LAC. – Stratum lacunosum
STR. MOL. (above HIP. SUL.) – Stratum moleculare of CA

HIP. SUL. – Hippocampal sulcus (vestigial)

Dentate Gyrus (DG) (bottom):

B: Granular soma
6. Apical dendrite of granular neuron
7. Basal of granular neuron (mossy fibre, to CA3/4)
14. Perforant path (from entorhinal cortex)
15. Commissural fibres
16. Septal fibres

Layers of the DG:
STR. MOL. (2/3 & 1/3) – Stratum moleculare of DG
STR. GR. – Stratum granulosum
POLY. LAY. – Polymorphic layer

(Duvernoy, 2005) (Appendix B)

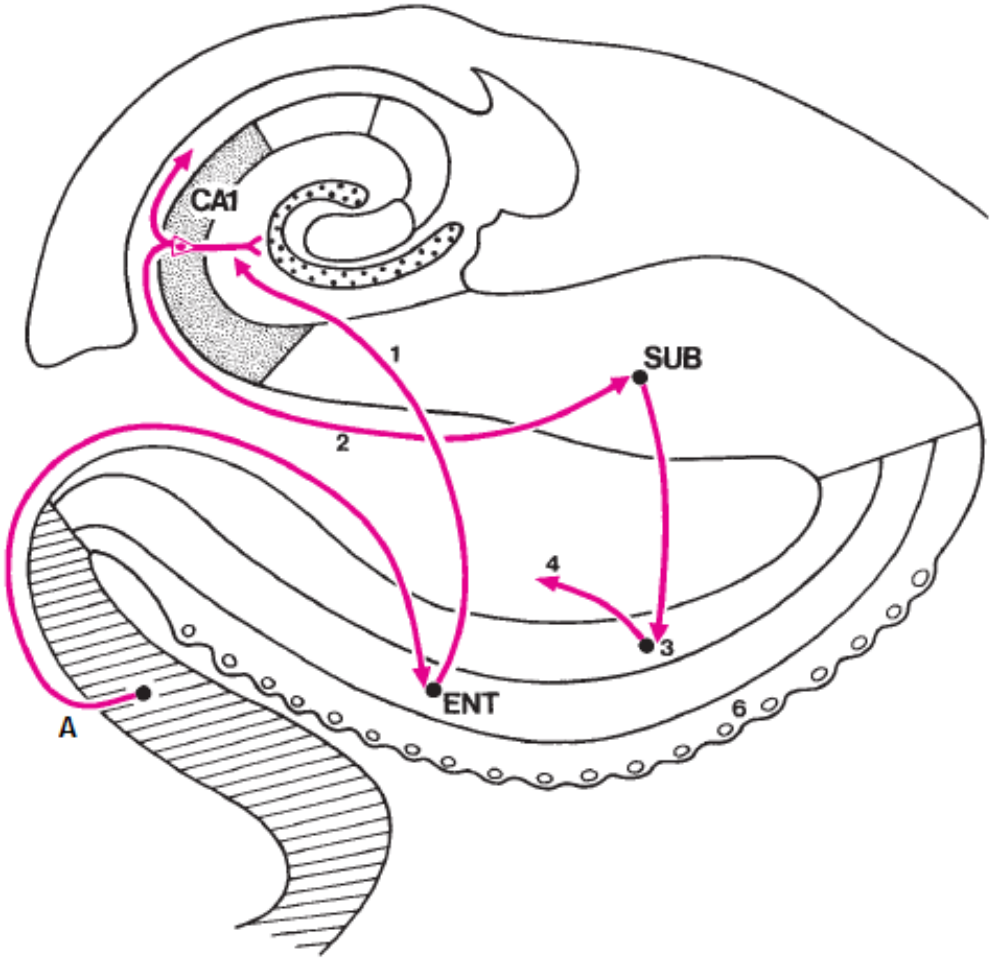

Figure A.3: The direct intrahippocampal pathway
A – Inputs through the perirhinal cortex (also see Figure A.4)
ENT – entorhinal area, layer III
SUB – Sub

1. Entorhinal area directly projecting to CA1 pyramidal neurons
2. CA1 pyramidal neurons' basal axons innervate Sub
3. Axons in the Sub return the projections to the deep layers of the entorhinal cortex
4. These neurons send signals to the association cortex (also see Figure A.4)

(Duvernoy, 2005) (Appendix B)

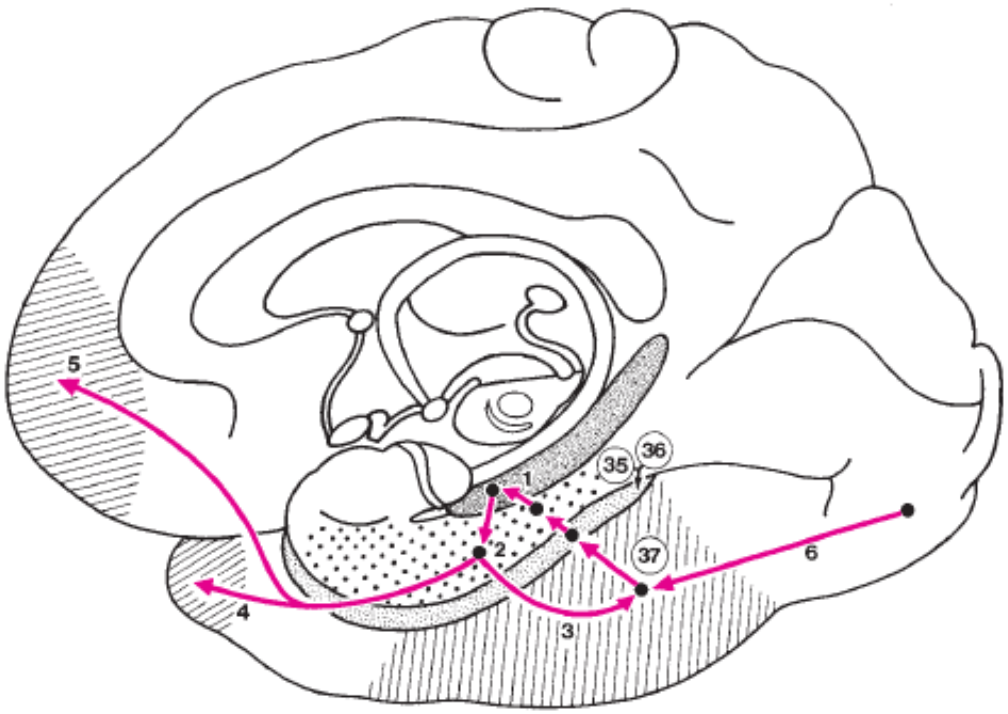

Figure A.4: Cortical connections to and from the direct intrahippocampal pathway

1. Intrahippocampal circuitry (also see Figure A.3)

INPUT:

6. Fibres from the inferior visual system to inferior temporal association cortex (area 37), connecting via the perirhinal cortex (areas 35, 36) to the entorhinal cortex

OUTPUT:

2. Fibres from the deep layers of the entorhinal cortex reach:
    3. The temporal association cortex
    4. The temporal pole
    5. The prefrontal cortex

(Duvernoy, 2005) (Appendix B)

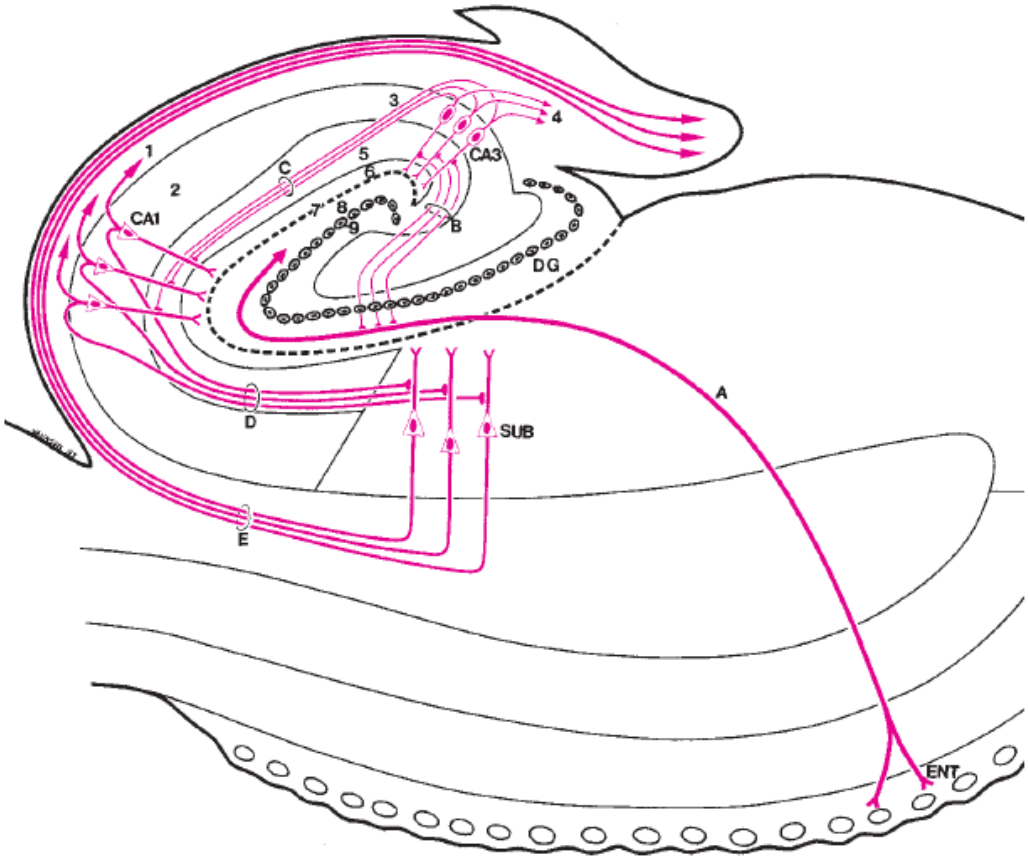

Figure A.5: The polysynaptic intrahippocampal pathway

1. Alveus; 2. Stratum pyramidale; 3. Schaffer collaterals;
   4. Axons of pyramidal neurons; 5. Strata lacunosum and radiatum;
   6, 8. Stratum moleculare; 7. Hippocampal sulcus (vestigial);
   9. Stratum granulosum

ENT – Layer II of entorhinal area; DG; CA1, CA3 – CA fields 1,3; SUB – Sub

A. Entorhinal cortex projects to the dentate gyrus (DG) through the Sub
B. The fibres from the DG cross over to stimulate the CA3 and CA4
C. In the CA3 and CA4, pyramidal neurons' Schaffer collaterals project to pyramidal neurons' apical dendrites in the CA1*
D. The CA1's pyramidal neurons send projections to the Sub*
E. The Sub's pyramidal neurons send the remainder of signals into the fimbria (also see Figure A.6)

* - Note: the CA fields also have some direct projections to the fimbria via the alveus

(Duvernoy, 2005) (Appendix B)

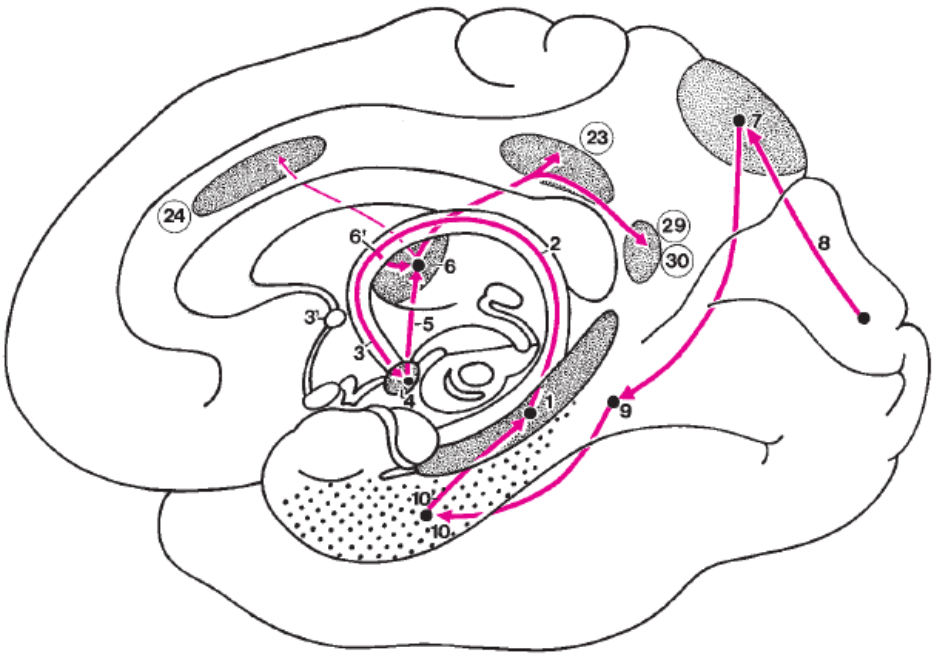

Figure A.6: Cortical connections to and from the polysynaptic intrahippocampal pathway

INPUTS:

8. Fibres originate from the superior visual system
7. Fibres project the posterior parietal association cortex
9. These fibres run through the parahippocampal gyrus
10. Fibres end up projecting to the entorhinal area via the perforant pathway (10')

(also see Figure A.5)

OUTPUTS:

1-3 Fibres reach and cross the fornix (just posterior to the anterior commissure (3')

4-6 Then, fibres run through the mamillothalamic tract (or directly) to the thalamus (6')

Then, from the anterior thalamic tract, they project mainly to:

Area 23: The posterior cingulate

Area 29, 30: The retrosplenial cortices

Area 24: The anterior cingulate cortex

(Duvernoy, 2005) (Appendix B)

Appendix B

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