

Non-Invasive Structural and Functional Connectivity of the In-Vivo Human Brain

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Abstract

Neuroimaging enables us to image the human brain and reveal information about its structure and function. Diffusion imaging infers anatomical white matter trajectories. Resting state functional MRI indirectly measures neural activity by determining Blood Oxygenation Level Dependent (BOLD) signal response at rest, inferring functional connections between brain areas. These independent neuroimaging modalities are advantageous due to their non-invasive and in-vivo nature, providing insight into the human connectome. Their relationship is yet not understood. We explored the structural and functional connectivity of the human brain at a macroscopic level, and the extent to which anatomical connections constrain functional connections. We analysed the correlations between functional and structural connectomes and found a strong correlation beyond the mere effect of distance between brain regions (Chapter 6). Next we delved into two different types of brain organisations - topographic and nuclear. Topographic connections between cortical areas are often ignored in macro-connectome models. We investigated such arrangements by modelling and simulating gradient of connectivity patterns as an example to encourage and bring awareness of their properties for future involvement into studying brain connectivity (Chapter 7). Finally we studied nuclear brain organisations in the human amygdala. The amygdala can be clustered into two main subdivisions, basolateral and centromedial. The basolateral complex consists of the lateral, basal and accessory basal subnuclei, whereas the centromedial complex comprises of the central and medial subnuclei. We utilised the two independent imaging modalities to parcellate the amygdala into its respective subnuclei in a data-driven approach using the Human Connectome Project (HCP) data. We studied the distinct afferent/efferent cortical projections of the human amygdala, and we parcellated the amygdala into its five subnuclei, anatomically and into two subdivisions, functionally (Chapter 8). Studying the structural and functional connectivity in-vivo and non-invasively has provided evidence of their similarities, at the cortical and subcortical level.

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Chapter 1

Introduction

The human brain is complex and is still a mystery to us today. Neuroscience intrigues scientists, from psychologists to mathematicians. One of whom, Thomas Willis, in the 17th Century, provided significant insight into our brain's anatomy and neurology. Over recent years imaging the brain is beginning to give us important information on the human connectome (i.e. the comprehensive map of brain connections). This thesis aims to explore non-invasive neuroimaging to study the structural and functional organisation of the human brain in the context of functional segregation versus integration (Chapter 6), and the different types of brain organisations present - continuous, topographic organisation in the cortex (Chapter 7), and clustered/nuclear type organisation in the amygdala (Chapter 8), both measured via in-vivo techniques.

The thesis comprises of the following chapters.

Chapter 2 covers neuroanatomy, from the microscopic to macroscopic level. The macroscopic level introductory material includes the cerebrum and its outer layer, cerebral cortex. We introduce brain connectivity in the context of white matter nerve tracts between brain regions, revealing insights into the human connectome.

Chapter 3 focuses on the principles of the non-invasive in-vivo neuroimaging technique, Magnetic Resonance Imaging (MRI). We commence by explaining nuclear magnetic resonance (NMR) and how this relates to MRI image formation from magnetisation, excitation and relaxation of spins. We compare the different types of imaging sequences used and their applications into structural and functional imaging.

Chapter 4 introduces diffusion imaging to reveal structural connectivity of the human brain. We describe how diffusion tensor model allows us to deduce local fibre orientation per voxel. We also cover tractography, the process of inferring trajectories of white matter fibres from diffusion data. We compare streamline and probabilistic tractography, and their applications.

Chapter 5 presents functional MRI (fMRI) in the context of functional connectivity of the human brain. We explain how changes in the brain relate to functional activity, measured as a Blood Oxygen Level Dependent (BOLD) signal and we outline how these developments determine brain activation at rest. We discuss experimental design, pre-processing and analysis techniques for optimisation of such imaging.

Chapter 6 explores the functional (functional MRI) and structural (diffusion) human connectome. Functional connectivity is thought to be a reflection of the underlying structural connectivity, though this is not entirely understood. Given the average structural and functional connectivity matrices, the functional-structural connectivity relationship between pairs of ROIs was calculated

using pairwise Pearson correlation coefficient. We observe the strongest correlation between an ROI's functional connectome is its own structural connectome, and distance plays a crucial role in cortical brain connectivity. Fitting an (Rational-Quadratic Model) RQM to each ROI regresses out the distance effect to observe similarities and differences between structural and functional connectivity for both the independent MRI techniques.

Chapter 7. Topography is a continuous type of cortical organisation defined as the point-to-point mapping between regions. We look into examples of topography and types, such as gradient patterns. We modelled and simulated such patterns as an example to learn how to potentially detect and classify these topographic connections, and to encourage inclusion of such organisations in future research studies.

Chapter 8 investigates the human amygdala nuclear organisation. The amygdala contains two subdivisions- centromedial and basolateral complex, which can be subdivided into five subnuclei- basal, accessory basal, central, medial and lateral. This chapter looks into the current methods of amygdala parcellation via diffusion or functional imaging, and adopts a data-driven approach using the Human Connectome Project (HCP) data to parcellate the amygdala, functionally and anatomically using the unique projections between the unique amygdaloid subnuclei projections to other brain regions.

Chapter 9 concludes the main findings of the thesis.

Chapter 2

Neuroanatomy

2.1 Terminology

A series of common terminologies are often adopted to describe the human anatomy. These standardised terms are derived to describe anatomical positions relative to one another; grouped in pairs to denote axes. The dorsal-ventral axis runs from top to belly, and rostral-caudal axis from head to tail, based on animal anatomy (Figure 2.1(a)). Our body is also visualised via planes running perpendicular to one another – the sagittal, coronal and axial (or transverse) plane (Figure 2.1(b)).

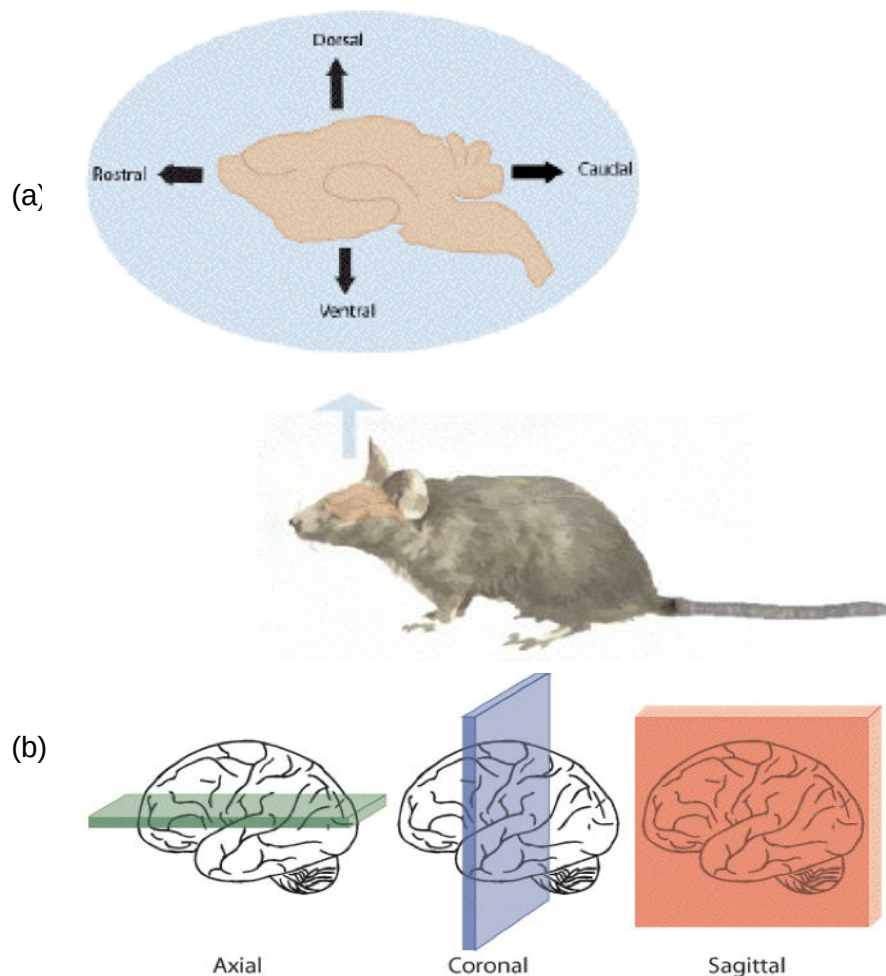


Figure 2.1. Principal anatomical axes and planes. (a) Anatomical directions are commonly used in anatomical terminology, where the dorsal-ventral axis runs from top to bottom and rostral-caudal from head to tail (b) Three perpendicular planes describe the body's anatomy- axial=midline division, coronal=superior-inferior division and coronal=anterior-posterior division. Figures taken from (Clare, 1997).

2.2 Microscopic Level

The central nervous system (CNS) comprises of neurons (or nerve cells) and glial cells (or neuroglia). The latter provides neural support, nutrients and oxygen supply, and insulation (Purves and Williams, 2001). Glial cells outnumber neurons in the CNS though recent studies suggest the glia-to-neuron ratio (GNR) varies depending on the brain region inspected (Hilgetag and Barbas, 2009). There are three main types of glial cells- astrocytes, oligodendrocytes and microglia. Astrocytes are star-shaped neuroglia identified by the expression of glial fibrillary acidic protein (GFAP) using

histology (Sofroniew and Vinters, 2010), and over recent years astrocytes are shown to be key in neurodevelopment and neurological disorders, such as epilepsy (Clasadonte and Haydon, 2012). Oligodendrocytes produce a fatty myelin sheath that wraps around parts of an axon for insulation and hence provide faster signal transmission (accomplished via Schwann cells in the peripheral nervous system (PNS)). The demyelination of such axon myelin sheath lead to neurodegenerative conditions, including Multiple Sclerosis (MS) (Prineas and Parratt, 2012). Finally, microglia are the smallest glial cells, derived from haematopoietic stem cells for neural protection and removal of CNS debris (Purves and Williams, 2001).

A neuron receives, processes and transmits neural signals from one neuron to other, composed of a single cell body (or soma), an axon (or nerve fibre), dendrites and axon terminals. The cell body includes the nucleus and other organelles, such as mitochondria, surrounded by the cytoplasm. The cell body processes neural signals, received by dendrites, and propagates it down the neuron's axon to its axon terminal as an action potential. An action potential is brought about by the balance and movement of electrically charged particles or ions (including sodium, potassium and chloride) via ion channels, located across an axon's cell membrane. Upon an action potential reaching an axon terminal's synaptic knob, chemical neurotransmitters such as glutamate, are released to the synapse for excitation or inhibition of the next neuron. Figure 2.2 illustrates the most common neuronal CNS type, multipolar neuron (one axons and many dendrites). Other neuronal types, such as Purkinje cell differ in shape and size, classified based on their structure and function (Masland,

2004). For instance, bipolar sensory neurons have two axonal projections extending from opposite ends of its cell body, found in sensory stimuli receptors, whereas unipolar neurons are made up of one neurite and commence as bipolar neuron during embryo neurodevelopment.

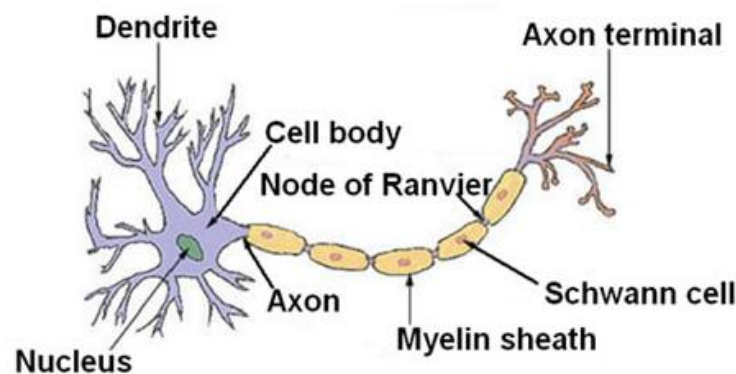


Figure 2.2. A neuron. A neuron is made up of a cell body, axon, dendrites, and axon terminals. The cell body processes inputted signals from the dendrite, resulting in action potentials propagating down an axon to the axon terminal. This excites or inhibits the next neuron by releasing neurotransmitters into the synapse. Taken from (Institute, 2013).

2.3 Macroscopic Level

At the organ level, the brain is divided into the brain stem, cerebellum and cerebrum, surrounded by the cerebrospinal fluid (CSF). The cerebrum consists of four lobes with distinct functions- the frontal, parietal, occipital and temporal lobe (Figure 2.3(a)). Firstly, the frontal lobe is the most rostral structure of the four, and involved in roles including reasoning, cognition, reward, problem solving and decision-making. Mental conditions such as Schizophrenia are associated with early motor and cognitive developmental changes (Murray et al., 2006). The parietal lobe is involved in spatial orientation and sensory information processing, with the somatosensory cortex situated in this lobe and somatotopically organised (Chapter 7).

Posterior to the parietal lobe is the occipital lobe, significant to the processing of visual information with discrete visual functional areas, retinotopically organised. The temporal lobe is located inferior to the frontal lobe and is associated with visual perception, memory and language.

Subcortical structures, the thalamus and basal ganglia are located deep within the brain (Figure 2.3(b)). The thalamus is positioned along the midline of each hemisphere, responsible for controlling and relaying sensory information to the cortex (Sherman et al., 2006). The thalamocortical connections between the distinct thalamic subdivisions (based on cytoarchitecture) and the outer layer of the cerebrum, cerebral cortex, are topographically organised (Morel et al., 1997). Recent studies show the parcellation of the thalamus into its nuclei using diffusion imaging (Behrens et al., 2003a; Johansen-Berg et al., 2005). On the contrary, the basal ganglia are a complex interconnected structure made of the striatum (putamen and caudate nucleus), subthalamic nucleus, globus pallidus and nucleus accumbens. The basal ganglia functions range from cognitive functions (Collins, 1998) to learning (Kimura, 1995).

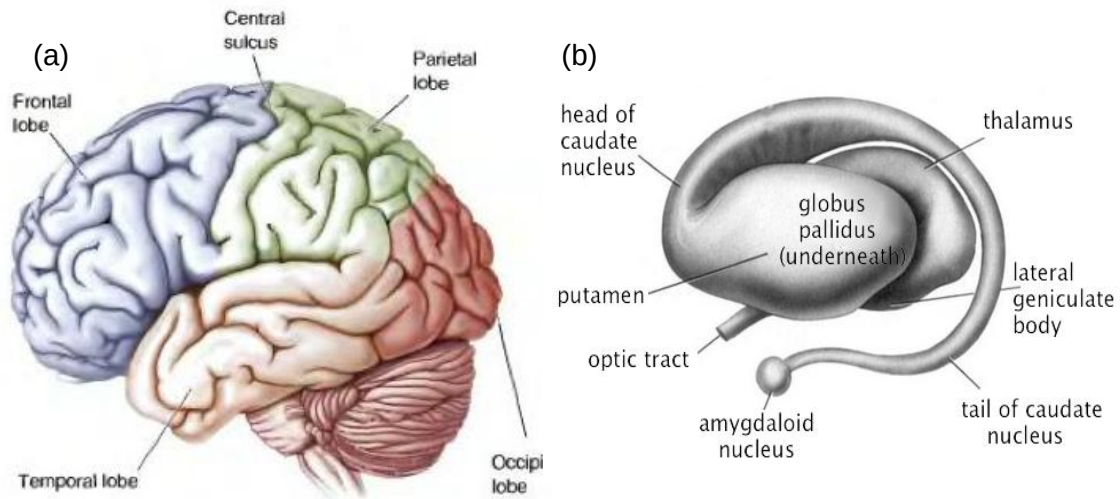


Figure 2.3. Cerebrum lobes and subcortical structures (a) The cerebrum is divided into four lobes: frontal, parietal, occipital and temporal. The frontal lobe is located at the front of the brain involved in reasoning, cognition, problem solving and decision-making. The parietal lobe is involved in sensory information processing. The temporal lobe is associated with emotion, memory and language. The occipital pole located at the back of the brain is significant in processing of visual information. Taken from (Bear et al., 2007) (b) The basal ganglia and thalamus are found deep in the cerebrum, located in the temporal lobe. The thalamus is involved in sleep and regulating sensory information to the cortex, and the basal ganglia functions entail motor control, reinforcement learning, cognitive functions, and learning. Taken from (Boeree, 2006).

2.3.1 Cerebral Cortex

The outer layer of the cerebrum, cerebral cortex (or neocortex) is made of grey matter, containing neuronal cell bodies and other neuronal parts, for instance dendrites. The collective functions of the cerebral cortex ranges from cognition to visual processing but it can be primarily divided into the sensory, motor and association areas.

The cerebral cortex is highly convoluted due to its gyri (bumps) and sulci (furrows), aimed at increasing surface area (Kandel et al., 2013). The temporal and frontal lobe is separated via the lateral fissure, whereas the central sulcus separates the frontal and parietal lobe. Deep within the lateral

fissure is the insular cortex, often separated from the frontal and temporal cortices, and involved in self-awareness, emotion, and pain perception (Nieuwenhuys, 2012).

The cerebral cortex is 2-4mm thick and organised into six distinct layers of discrete neural types, density and organisation (Figure 2.4) (Shipp, 2007). Layer I (molecular layer) is the most superficial layer containing neuroglia and a low level of scattered neural cells. One type of neuronal type, the star-shaped stellate cells (inhibitory interneurons) are found in the molecular layer, characterised by several dendrite projections radiating from the soma. Layer II (external granular layer) contains small granular cells, stellate cells and pyramidal cells. Pyramidal neuron's name was derived from its pyramidal shaped soma, two separate dendrite projections and single axon (Spruston, 2008). These excitatory cells are also found in other subcortical structures, such as the amygdala and prefrontal cortex implying roles in neurocognition (Elston, 2003). Layer III (external pyramidal layer) primary consists of two sub-layers of pyramidal neurons. Layer IV (internal granular layer) is a combination of pyramidal and stellate cells with thalamocortical input afferent connections. Layer V (internal pyramidal layer) is made of large pyramidal neurons with afferent projections to subcortical structures from the spinal cord and brain stem. Lastly, Layer VI (multiform layer) containing various neural cells, including a small quantity of pyramidal neurons. Efferent and afferent, excitatory and inhibitory projections to the thalamus are expressed in this layer. Overall, these six cortical layers are arranged in cortical columns.

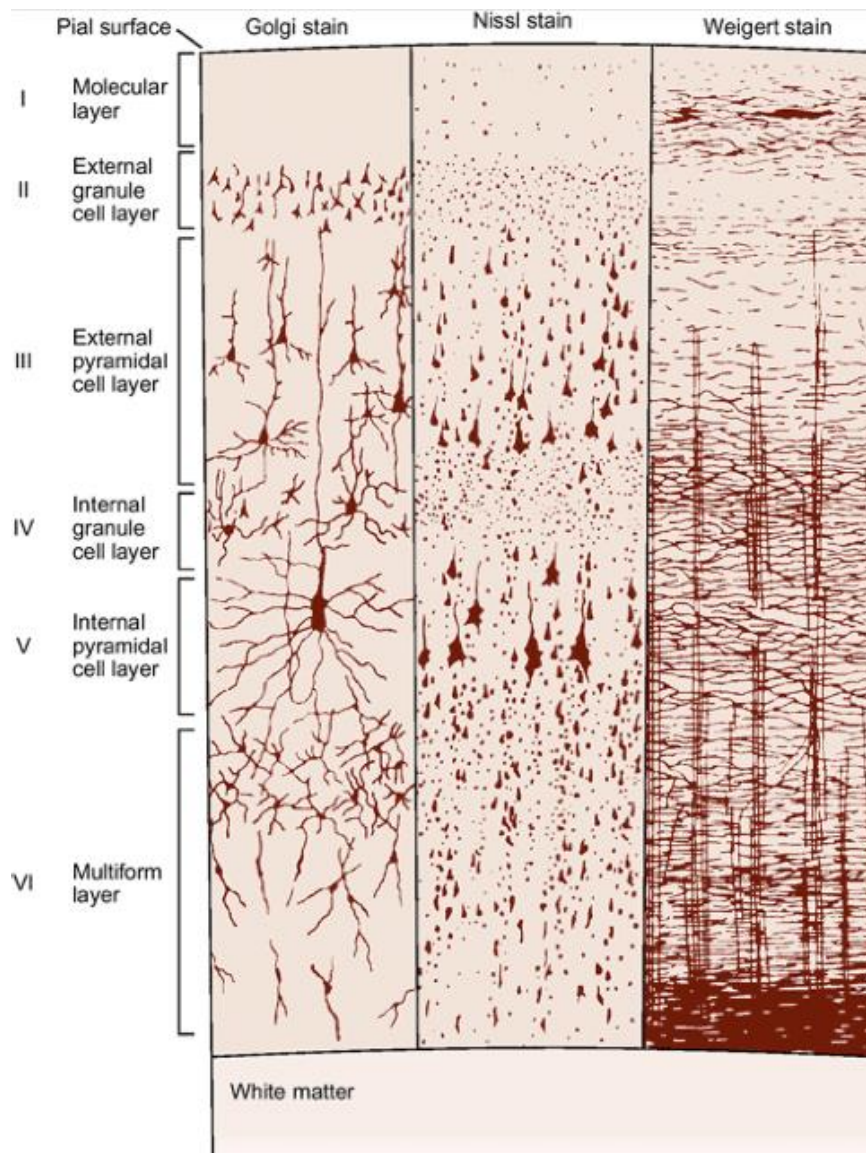


Figure 2.4. Cerebral cortex's cortical layers. The cerebral cortex is organised into six cortical layers with its visualisation dependent on staining techniques used, ranging from Golgi stain to Nissl stain. In order of superficial to deep layer – Layer I: molecular layer, Layer II: external granular layer, Layer III: external pyramidal layer, Layer IV: internal granular layer, Layer V: internal pyramidal layer and Layer VI: multiform layer. The layers are arranged in cortical columns. Taken from (Kandel et al., 2000).

2.3.2 Brain Connectivity

White matter comprises mostly of myelinated axons, where a group of fibres that follow similar paths form tracts to communicate between grey matter areas. These tracts fall into three categories- commissural, projection and association tracts, illustrated in Figure 2.5 (Catani et al., 2002). The cerebrum

is divided into the left and right hemispheres communicating via the commissural tracts, such as the corpus callosum. Projection tracts are made of afferent and efferent projections, linking the lower parts of the brain and spinal cord with the cerebral cortex. Lastly, association tracts connect cortical regions of the same hemisphere and association areas, such as the inferior longitudinal fasciculus (ILF) connecting the occipital and temporal pole together.

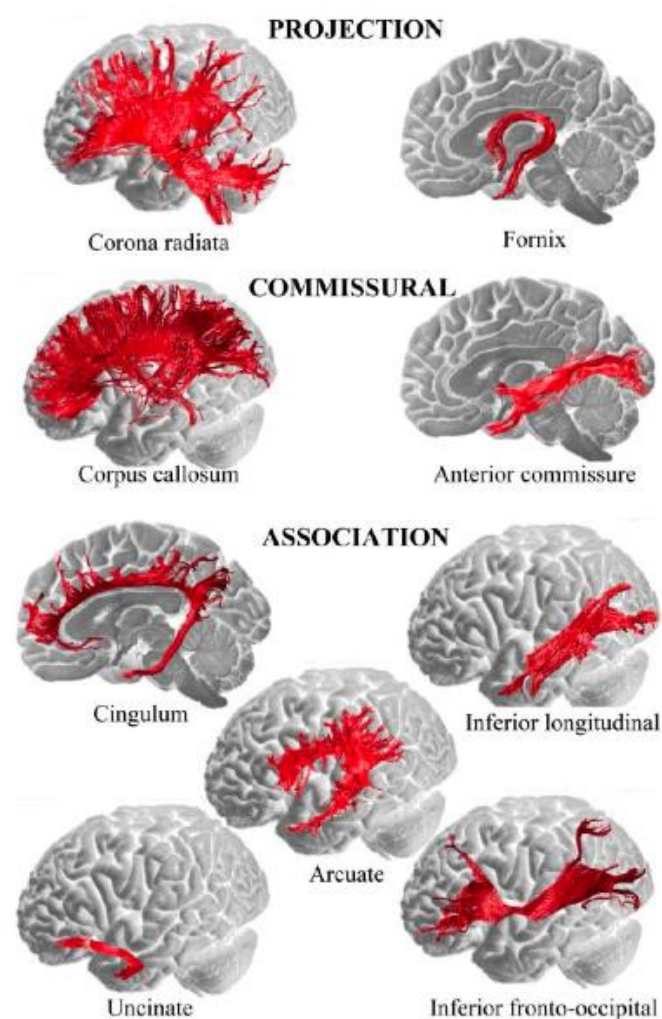


Figure 2.5. White matter tracts. There are three main types of white matter tracts. Projection tracts connect the lower parts of the brain and spinal cord with the cerebral cortex. Commissural tracts enable communication between the right and left brain hemisphere. Association tracts connect the brain regions on the same hemisphere. Taken from (Catani and ffytche, 2005).

The physical interactions between neurons or regions, result in structural connections that run over several scales, from microscale to macroscale. The mapping of anatomical tracts are referred to as the connectome (Van Essen and Ugurbil, 2012), and the human connectome aims to map the large scale human brain connections (Van Essen et al., 2013). Current methods of investigating the structural connectome include dissections, histological tracers and neuroimaging. The latter, includes diffusion imaging and functional MRI (fMRI). Diffusion imaging is an in-vivo and non-invasive imaging technique that determines structural connectivity from axonal projections, whereas functional connectivity, deduced via functional MRI (fMRI), enables us to investigate the neural activity between regions. These imaging modalities and long-range interactions are the focus of this thesis covered in more detail in the following chapters.

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Chapter 3

MRI

3.1 Introduction

Neuroimaging entails the use of diverse techniques to image the human brain.

Electrophysiology (magnetoencephalography (MEG) and electroencephalography (EEG)), radiography (X-Rays) and Magnetic Resonance Imaging (MRI) are all methods aimed at measuring, directly or indirectly, the different properties of the brain (Figure 3.1).

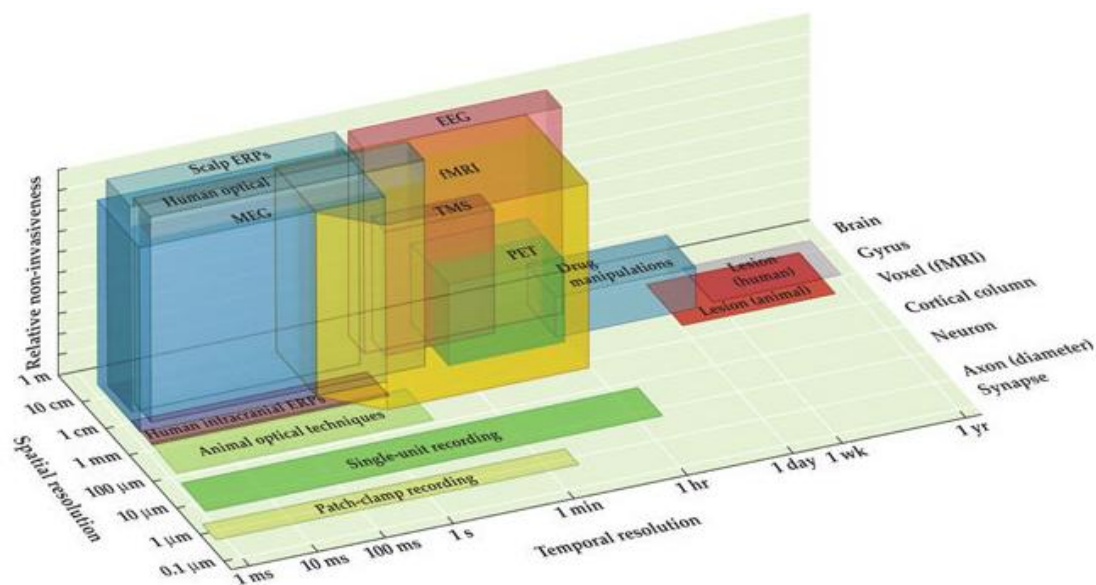


Figure 3.1. Comparison of temporal, spatial and relative non-invasiveness techniques for probing human brain activity. MEG has high temporal resolution though expensive. PET is of limited temporal resolution and relatively less non-invasiveness. MRI (including fMRI) is non-invasive and offers good temporal resolution. Taken from (Huettel et al., 2004).

Invented in the 1970s, MRI is a powerful in-vivo neuroimaging tool for studying the structural and functional properties of the human brain, introduced by Paul Lauterbur (Lauterbur, 1989). Based on the use of magnetic fields and radio waves, we can produce images of internal properties (i.e. soft tissue) by utilising their magnetic properties. In general, MRI is expensive, but non-invasive, offers good soft tissue contrast and does not involve any ionising radiation, compared to other imaging techniques such as PET. This chapter delves into the principles of MRI.

3.2 Magnetisation

Magnetic resonance was once referred to as Nuclear Magnetic Resonance (NMR), first described by Felix Bloch and Edward Purcell in 1946 (Bloch, 1946; Purcell et al., 1946). Magnetic resonance only affects the atomic nucleus described by its charge, mass and spin angular momentum. The latter, spin angular momentum is a fundamental physical property characterised by the spin quantum number, s that holds discrete values of half integers (Buxton, 2009), and a spin is randomly orientated under the presence of no magnetic field with zero net magnetic moment (Figure 3.2(a)). However, under an external strong main magnetic field, B_0 applied along the z-axis and typically between 1.5 and 7 Tesla (T) ($1\text{T}=10,000$ Gauss), spins parallel to B_0 at a lower energy state are greater than those antiparallel (higher energy state) with an energy difference, ΔE between both states, given by Equation 3.1, where h is Planck's constant (6.6×10^{-34} Js) and γ is the gyromagnetic ratio (Table 3.1). Thus, antiparallel spins cancel out with parallel spins and the remainder Net Magnetisation Vector, M_0 determines the total spin magnitude

vector in the B_0 direction (Figure 3.2(b)). Furthermore, a spin can be represented as a rotation about an axis and at B_0 , M_0 experiences a torque causing it to precess about the magnetic field at a Larmor frequency, ω , called the Larmor precession, given by Equation 3.2 and illustrated in Figure 3.3.

$$\Delta E = \frac{h\gamma B_0}{2\pi} \quad (3.1)$$

$$\omega = \gamma B \quad (3.2)$$

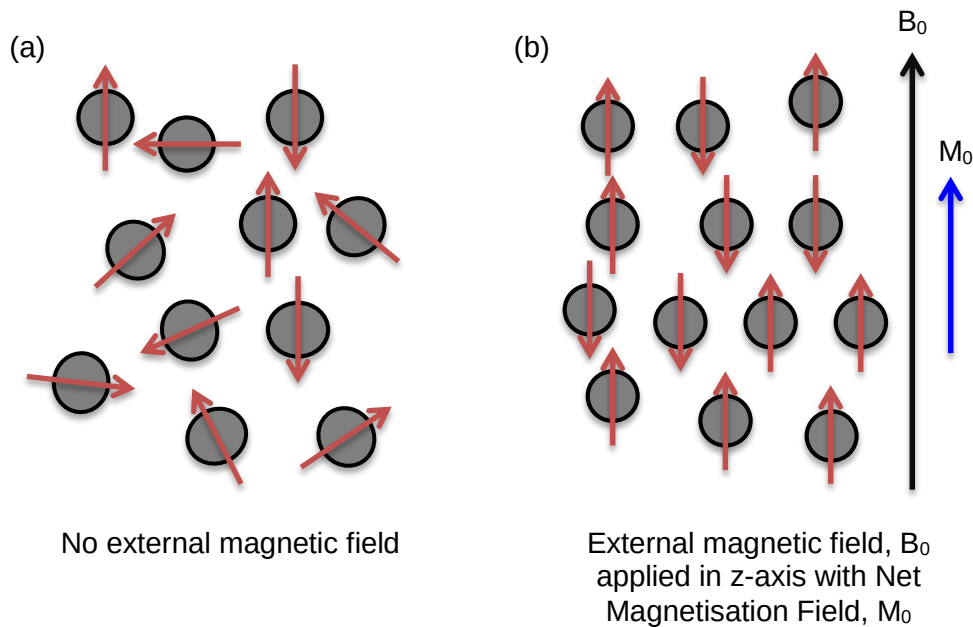


Figure 3.1. Spins with and without magnetic field presence (a) Spins are randomly orientated under no presence of an external magnetic field, resulting in a zero net magnetic moment (b) Under an external magnetic field, B_0 applied in the z-axis, more spins are aligned parallel to B_0 than antiparallel with an average net magnetisation vector, M_0 .

Table 3.1. Gyromagnetic ratios are different for different nucleuses (Buxton, 2009).

Nucleus	γ , Gyromagnetic ratio (MHzT ⁻¹)
¹ H	42.58
¹³ C	10.71
²³ Na	11.27
³¹ P	17.25

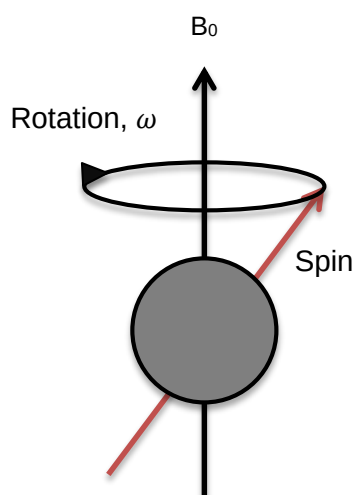


Figure 3.3. Larmor precession. Spins precess about an external magnetic field, B_0 at the Larmor frequency, ω . This is called Larmor precession (Equation 3.2).

3.3 Excitation and Relaxation

We can interact and alter nuclear spin system properties by exciting spins, tuned to the Larmor frequency. This excitation is brought about via an alternative magnetic field, often applied as an oscillating electromagnetic (EM) wave, perpendicular to B_0 and referred to as a radio frequency (RF) pulse, denoted by B_1 . The RF pulse tips the net magnetisation vector away from its equilibrium state, by a flip angle, α (Equation 3.3), where γ is gyromagnetic ratio (42.58 MHzT⁻¹ for hydrogen) and τ is the RF pulse length.

$$\alpha = \gamma B_1 \tau \quad (3.3)$$

At a 90 degree RF pulse, M_0 is excited and tipped from its preferred state parallel to B_0 , where there is only longitudinal magnetisation, M_z , to its higher energy state of only transverse magnetisation, M_{xy} or M_T , observed using a receive coil located perpendicular to M_{xy} . Once the RF pulse is switched off the net transverse magnetisation vector slowly relaxes to its lower energy state by emitting energy to the surroundings tissue (lattice) in a process called relaxation. The time constant of relaxation is called the longitudinal relaxation time (T_1) or spin-lattice relaxation, governed by Equation 3.4. T_1 describes the exponential time it takes for M_z to revert back to its equilibrium, typically varying between 100-1000ms depending on tissue type (Vlaardingerbroek and Boer, 1999).

$$M_z = M_0(1 - e^{\frac{-t}{T_1}}) \quad (3.4)$$

Spins are also influenced by small magnetic fields of neighbouring spins causing them to precess at slightly different Larmor frequencies to one another (Abragam, 1961). This results in spin dephasing and fanning out (Figure 3.4) (Hahn, 1950), described by spin-spin relaxation time, T_2 (shorter than T_1) defined as the time, t , for the transverse magnetisation to exponentially decay given by Equation 3.5. The slow decay of NMR signal over time due to relaxation effects is called Free Induction Decay (FID).

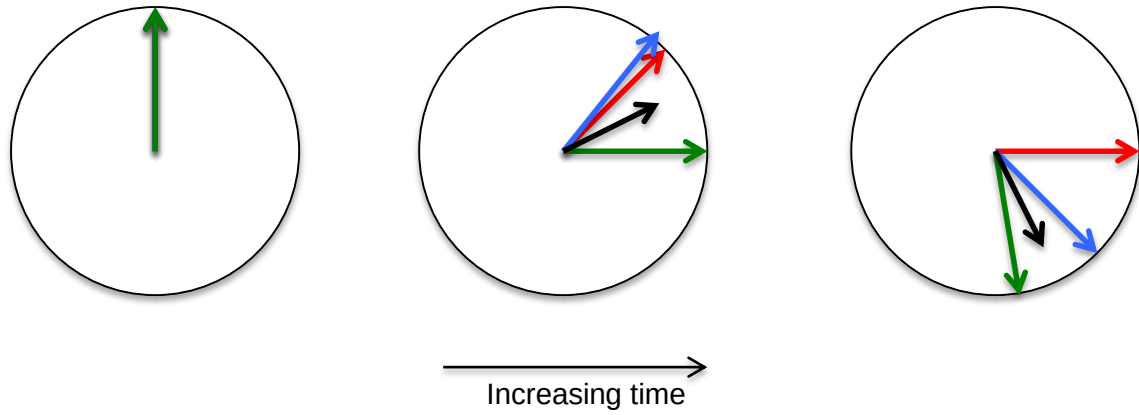


Figure 3.4. Dephasing of spins over time. Initially the spins are aligned together in phase. Once an RF pulse is switched off, spins start precessing at different frequencies causing them to fan out. Red, blue and green refer to the different spins, and black represents the net magnetic moment, M_0 .

$$M_{xy} = M_0 e^{\frac{-t}{T_2}} \quad (3.5)$$

Ideally, the transverse magnetisation vector decays and longitudinal magnetisation vector recovers, although other factors such as susceptibility effects and B_0 inhomogeneities cause extra dephasing effects, characterised by the T_2^* decay constant. The relationship between T_2 and T_2^* is given by Equation 3.6, where $\gamma\Delta B_0$ refers to the difference in strength of locally varying field. Chapter 5 highlights the importance of T_2^* in fMRI imaging.

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \gamma\Delta B_0 \quad (3.6)$$

3.4 Gradient Echo and Spin Echo Imaging

Relaxation time constants are valuable in distinguishing between tissue types (grey matter, white matter and CSF) but acquisition parameters are key in MR measurements. Echo Time (TE), the time between RF pulse application and maximum echo MR signal, and Repetition Time (TR), the time between

successive excitation RF pulses (determining temporal resolution) are significant in constructing MR images. A longer TE, associated with greater T_2 relaxation results in a T_2 -weighted image, whereas a shorter TR, less than T_1 is referred to as T_1 -weighted MR image. Furthermore, a shorter TR causes a lower longitudinal magnetisation, M_z as the signal does not relax back to equilibrium state before being re-excited at its next RF pulse, compared to that of longer TR (Figure 3.5). For instance at 1.5T, white matter T_2 is 70ms and CSF T_2 is 400ms (Buxton, 2009). For short TR, white matter would appear brighter compared to CSF due to its greater recovery time (brighter image) and MR signal.

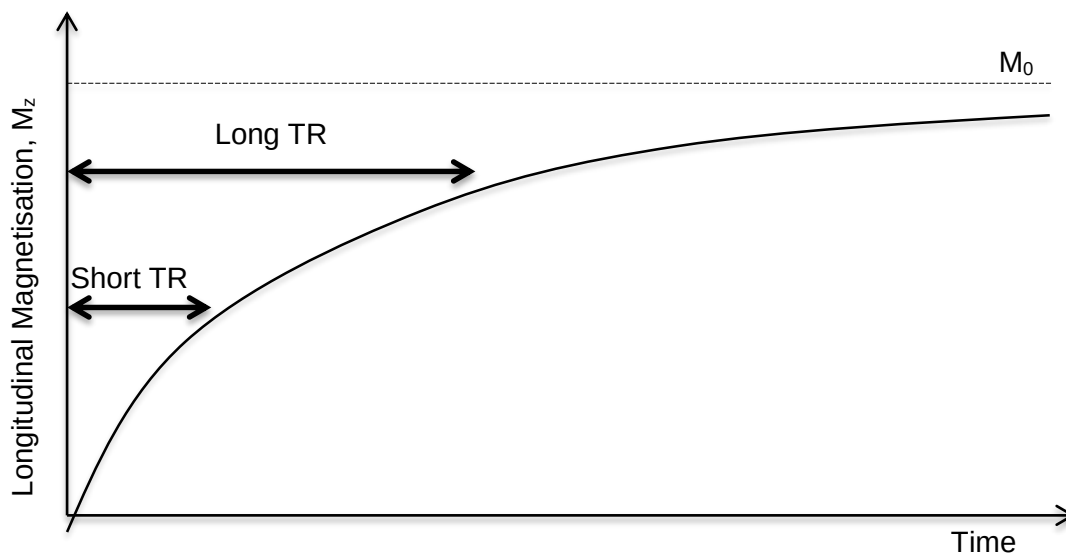


Figure 3.5. Longitudinal magnetisation over time for different repetition time (TR). TR is the time between two RF pulses. At short TR spins do not completely returning back to their preferred energy state parallel to B_0 before being re-excited by the next RF pulse, compared to a long TR. This enables tissue contrast between white matter, grey matter and CSF.

A pulse sequence is a pattern of RF pulses (applied at fixed TR's times) and gradient pulses generating an MR signal. There are different types of pulse sequences, such as spin-echo (SE) and gradient echo (GE) Echo Planar

Imaging (EPI) sequences. The different types of pulse sequences available have different advantages and disadvantages, but all follow the same principles of varying acquisition parameters to affect MR signal outcome and the image constructed.

A schematic of an SE sequence is shown in Figure 3.6 (Tanner and Stejskal, 1965). A 90 degrees RF pulse is applied to flip the magnetisation vector onto the transverse plane that dephases over time (Hahn, 1950). Edwin Hahn rectified this dephasing drawback by applying a secondary 180 degrees RF pulse at $\frac{TE}{2}$ resulting in spin rephasing (Figure 3.7) (Hahn, 1950). The data is acquired after the spins are in phase at maximum MR signal given by time TE.

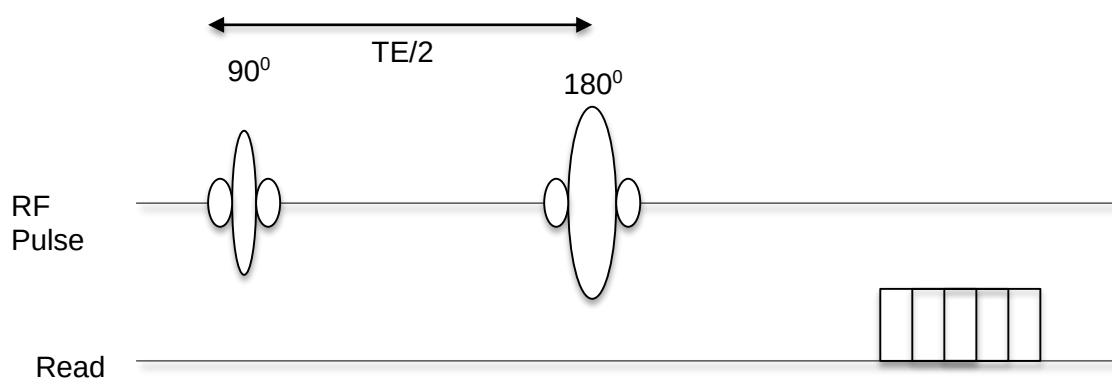


Figure 3.6. Spin-Echo (SE) sequence. An RF pulse tilts the spins onto the transverse plan that dephases spins over time but a second RF pulse at TE/2 rephrases the spins. Magnetic field gradients are later applied for localisation and acquisition.

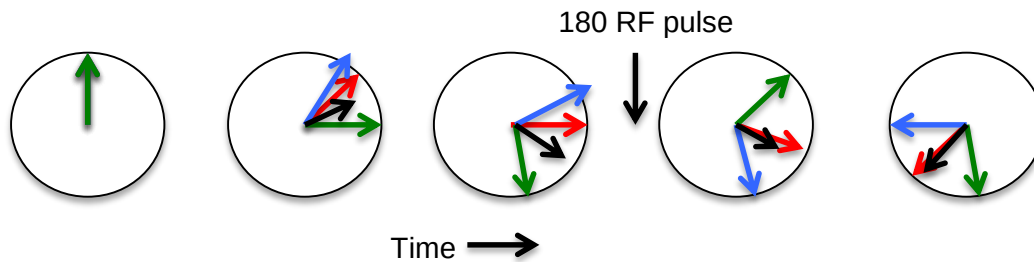


Figure 3.7. Dephasing and rephasing of spins (left to right). Immediately after an RF pulse is applied the spins are aligned together in phase. Over time the spins precess at slightly different frequencies causing them to fan out. After the application of an 180 degrees RF pulse the spins lagging come ahead and those ahead go behind resulting in rephasing of spins for signal acquisition at echo time, TE. Red, blue and green refer to the different spins, and black represents the net magnetic moment, M_0 .

SE is popular in diffusion MRI as it is less sensitive to susceptibility artefacts and provides good contrast, though GE sequence is commonly used in fMRI due to its higher image quality, speed and sensitivity to Blood Oxygenation Level Dependent (BOLD) signal. GE does not include a 180 degrees RF pulse for refocusing of FID, but rather a frequency encode gradient (Figure 3.8). The first frequency encode gradient is applied in reverse to speed up dephasing of spins, followed by another gradient frequency encode of same magnitude and duration but in the opposite polarity to rephase the spins. As a result of this the scanning time is reduced because there is no secondary RF pulse. GE allows for a flip angle below 90 degrees, decreasing the net magnetisation vector tipped onto the transverse plane, increased recovery speed of the longitudinal magnetisation and hence faster fMRI data acquisition with shorter TR.

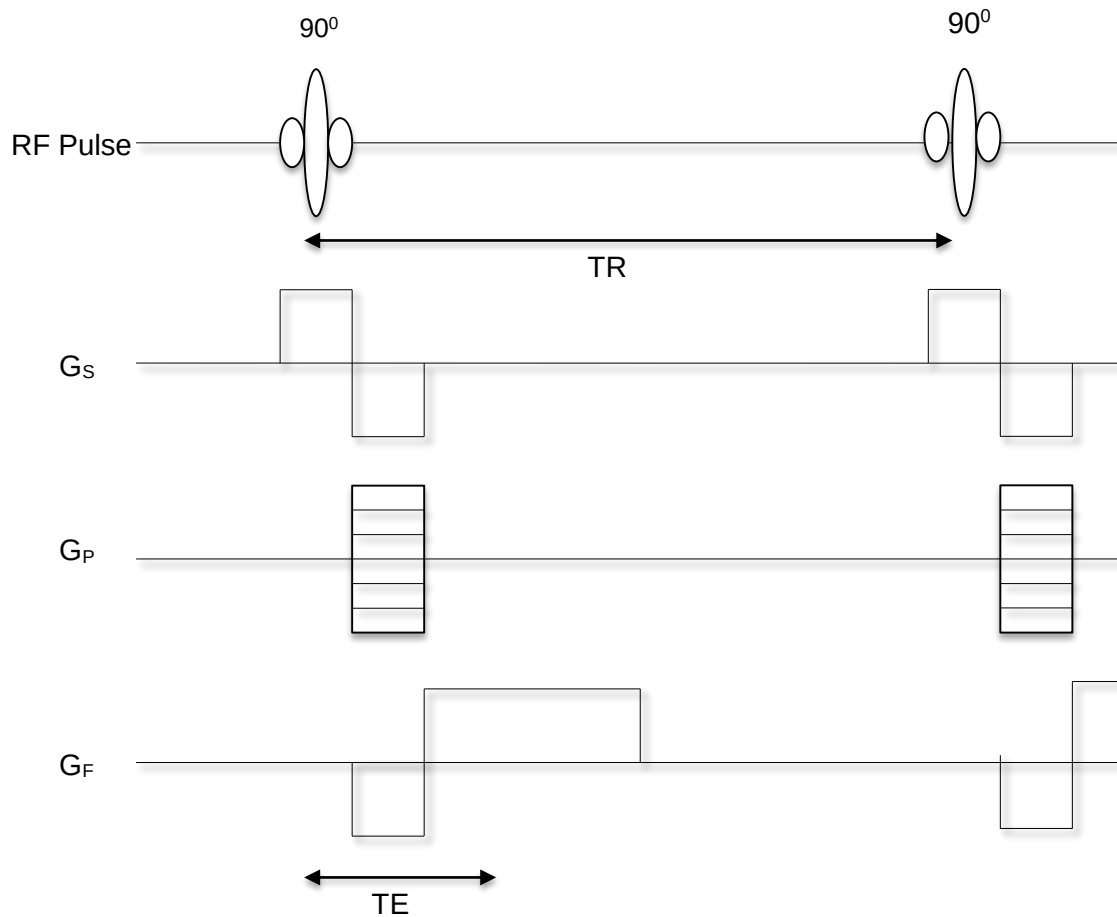


Figure 3.8. Schematic of Gradient Echo (GE) Sequence. An RF pulse tilts the spins onto the transverse plane that dephases spins, but no 180° RF pulse is added compared to SE EPI for rephasing of spins. A single frequency encode gradient (G_F) speeds up dephasing of spins, and another gradient frequency encode in the opposite direction but of same magnitude rephases the spin. This reduces scanning time. The slice selection gradient (G_S) excites spins in a single plane at a given time, whereas phase encoding (G_P) refers to a gradient field along the y-axis resulting in different precession of spins in the y-axis.

3.5 Image Formation

A magnetic field gradient is applied in a pulse sequence to localise spins for image formation. Gradients are applied along an orthogonal direction (x, y and z) and linearly dependent along those directions. Turning on combinations of the magnetic gradient field can generate any direction, alter frequency precession and provide spatial information. Such localisation involves slice selection, frequency encoding and phase encoding.

Slice selection excites spins in a single plane at a given time. Whilst an RF pulse is on, a gradient field along the slice selection axis is superimposed, therefore only the spins at the Larmor frequency that matches the RF pulse frequency will be excited and detected. In practice, there is a range of frequencies with an RF pulse bandwidth, BW_{RF} determining the width of the slice selected. Equation 3.7 is the slice thickness on the z-axis, where G_z is the gradient in the z-direction, assuming that is the z-axis by which B_0 exists.

$$\Delta z = \frac{BW_{RF}}{\gamma G_z} \quad (3.7)$$

An MR image is also dependent on frequency and phase encoding. The read-out frequency encoding gradient field, G_x encodes spins in the x-direction. The precession frequency is a function of position, x applied during signal acquisition and the signals associated with each frequency can be filtered out. The signals are converted into a collection of amplitudes associated with different frequencies by computing the Fourier Transform (FT). On the other hand, phase encoding occurs before signal acquisition and correspond to a gradient field in the y-axis, G_y applied for a short duration between the RF pulse and data acquisition, resulting in different precession of spins in the y-axis. Phase encoding compares the phase of the spins of same frequency to provide insight into spatial information.

Slice selection, phase encoding and frequency encoding are used to form an MR image by applying a FT of the imaging volume in Fourier space, called k-space data. The k-space rows refer to phase encoding and the columns to

frequency encoding, whereby each step along a row corresponds to one phase-encoding step. Once the k-space matrix is achieved its FT is computed to generate a presentable MR image.

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Chapter 4

Diffusion MRI

4.1 Diffusion

Consider a glass of water placed on a table. At first glance the water appears stationary, no movement, though at the molecular level, molecules are in constant random motion colliding with one another. At non-zero absolute temperature, molecules are in constant motion due to heat absorption. This was coined Brownian motion after Robert Brown, who in the early 1800s observed the random thermal motion of pollen grain in water under a microscope (Brown, 1828). Now consider the same glass of water on the table but a drop of fluorescent dye is added. Initially the dye is concentrated but over time the molecules diffuse from a region of high concentration to low concentration, radially, until reaching equal uniform concentration. Diffusion is an example of a transport phenomenon and was mathematically described by Adolf Fick in 1855 as Fick's First Law, Equation 4.1 (Fick, 1855a; Fick, 1855b). This law states that the flux of particles J is directly proportional to the concentration gradient, ∇C . The proportionality constant, D is called the diffusion coefficient or diffusivity.

$$J = -D\nabla C \quad (4.1)$$

Another, more statistical formulation of the diffusion phenomenon emerged at the beginning of the twentieth century, describing the motion of an ensemble

of particles. Any one molecule in the glass of water will undertake a 'random walk' independent of other molecules, with trajectories that are impossible to predict. However, in 1905 Albert Einstein described the distribution of displacement of an ensemble of molecules under a homogenous barrier free medium, Equation 4.2 (Einstein, 1905). Einstein discovered that, over time, t the mean squared displacement of particles $\langle x^2 \rangle$ is proportional to time, where D is diffusion coefficient. This is useful for determining the time it takes for molecules to diffusion over a certain distance, for instance the time it takes for chemicals (such as neurotransmitters) to diffuse across the synaptic cleft of 20-50nm from the presynaptic to the postsynaptic membrane.

$$\langle x^2 \rangle = 2Dt \quad (4.2)$$

In general, diffusion in biological tissues is not 'free' but is restricted by obstacles, such as membranes and other cellular processes. In white matter, molecules diffuse with more ease along myelinated axons than across them. Einstein's equation allows us to relate the amount of displacement over time to the diffusion coefficient. This is called the Apparent Diffusion Coefficient (ADC). Measuring ADC is the goal of diffusion MRI (dMRI) (Le Bihan et al., 1986), a non-invasive imaging technique brought about in the mid-1980s that allows us to estimate ADC of water molecules and hence infer brain tissue microscopic organisation (Le Bihan and Breton, 1985).

4.2 Imaging diffusion

As explained in Chapter 3, a spin is a fundamental property of a nucleus and forms the basis of measuring diffusion MRI accomplished via a Spin-Echo EPI sequence (Figure 3.6) (Tanner and Stejskal, 1965). A 90 and 180 degree RF pulse is applied to tilt the magnetisation vector onto the transverse plane, resulting in dephasing and later rephasing of spins (Hahn, 1950). Stronger gradients cause a stronger change in phase and increased diffusion sensitivity, called b-value or factor, governed by Equation 4.3, where γ is gyromagnetic ratio, G is the gradient magnitude, δ is gradient duration and Δ is gradient separation or diffusion time (Tanner and Stejskal, 1965).

$$b - value = \gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3} \right) \quad (4.3)$$

In general, diffusion imaging is dependent on gradient strength and orientation, and diffusion time. An increase in b-value results in increased sensitivity, though decrease in signal. The decrease in MR signal attenuation is governed by Equation 4.4, where S_1 is the signal intensity after diffusion encoding, S_0 is signal intensities without any diffusion encoding, and ADC is the apparent diffusion coefficient (Johansen-Berg and Behrens, 2009a; Le Bihan et al., 1986; Tanner and Stejskal, 1965).

$$S_1 = S_0 e^{-b.ADC} \quad (4.4)$$

4.3 Modelling Orientation

As explained previously, magnetic field gradients can be applied along any direction by using combinations of gradients along orthogonal directions. In diffusion, we acquire data in a range of directions and use this information to infer hindrance of water molecules. For instance, in the corpus callosum there is greater signal attenuation when the gradients are applied along the x-axis compared to the y-axis, hence inferring that bundles are aligned along the x direction (Figure 4.1). By fitting models to the diffusion signal, we can deduce tissue orientation, assuming diffusion is less hindered along axonal bundles. It is worth noting that whilst axons differ in length and diameter, typically of a few micrometres wide in the human brain (Lodish, 2000), in diffusion imaging, voxels are of a few millimetres cubed. Therefore we can infer principle tissue orientation of the axon bundles and not individual nerve fibres. We describe the diffusion tensor model, which is the most commonly used model in dMRI.

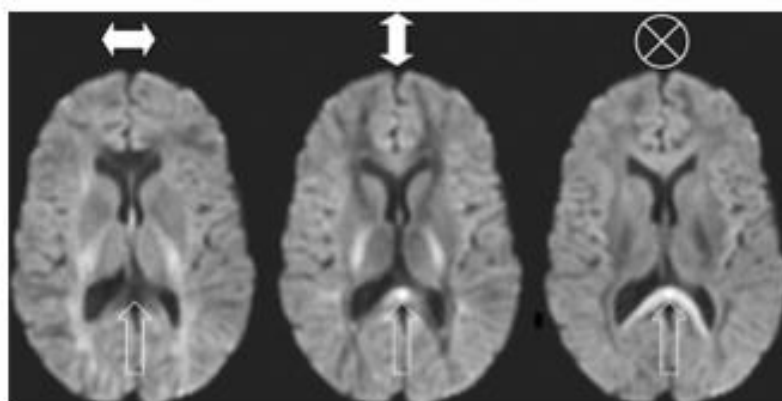


Figure 4.1. Different gradient directions applied to the human brain. The corpus callosum shows greater signal attenuation along the x-axis compared to the y- or z-axis, inferring that bundles are aligned along the horizontal plane. Taken from (Johansen-Berg and Behrens, 2009b).

4.3.1 Diffusion Tensor

From Equation 4.4, we need at least two different b values to determine ADC. At $b=0$, we are insensitive to the diffusion process as $S_1=S_0$ resulting in a non diffusion-weighted image, however at $b>0$ we obtain a diffusion weighted image, such that the signal intensity is less than S_0 . Therefore ADC is derived as

$$ADC = \frac{-\ln(S_1/S_0)}{b_1 - b_0} \quad (4.5)$$

In isotropic tissue, such as cerebrospinal fluid or grey matter, ADC is constant along all orientations (isotropic diffusion), though in white matter, ADC is anisotropic. The diffusion tensor model was introduced in the 1990s to model such diffusion (Basser et al., 1994), assuming diffusion within tissues follows a Gaussian distribution implying that at each voxel the diffusion signal obeys the following equation

$$S_j = S_0 e^{-b_j x_j^T D x_j} \quad (4.6)$$

where S_j is the signal measured with gradient along direction x_j (unit vector), b_j is the b -value associated with gradient j , S_0 is the signal at $b=0$ (the non-diffusion-weighted image) and D is the diffusion tensor. Rather than a scalar diffusion coefficient, the model uses a tensor, D , a 3x3 symmetric matrix that describes anisotropic diffusion in three-dimensions.

$$D = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix} \quad (4.7)$$

The diagonal elements of the matrix D are representative of ADCs along the three orthogonal axes and off-diagonals are proportional to the correlations (covariance) of displacement along these orthogonal directions.

A convenient way to analyse and visualise the diffusion tensor is by using a reference frame, called eigensystem and shown in Figure 4.2. The three eigenvalues correspond to the diffusivities along the principal orthogonal axes of the diffusion tensor (Basser et al., 1994).

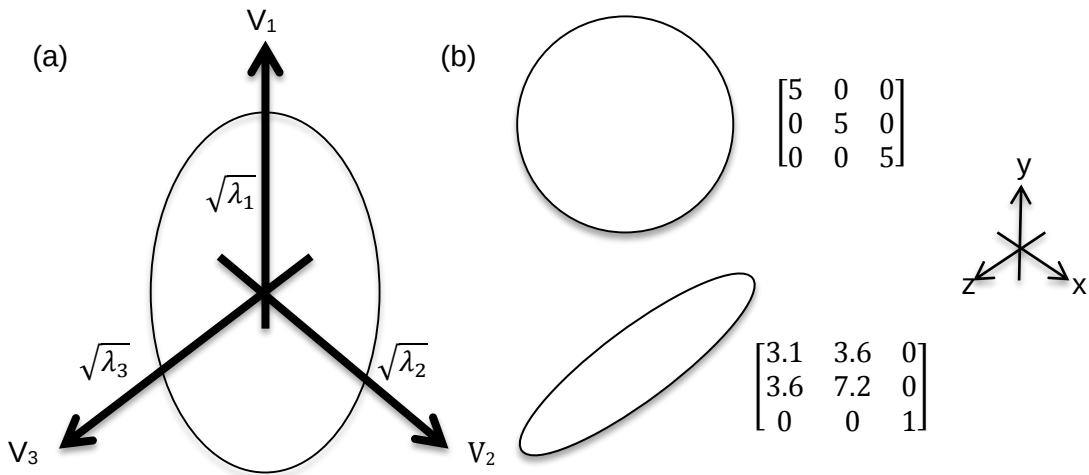


Figure 4.2. Eigensystem. (a) Diffusion tensor model with eigenvalues, λ_i and eigenvectors, V_i to an ellipsoid fitted to each voxel along three orthogonal directions (b) Examples of the diffusion model matrix, D of an isotropic and anisotropic model.

Equation 4.8 computes the degree of anisotropy. Fractional anisotropy (FA) (Equation 4.9), defined as the normalised eigenvalues variance is a metric for measuring diffusion anisotropy ranged between 0 and 1, where 0 is perfectly

isotropic as $\lambda_1=\lambda_2=\lambda_3$ (grey matter) and 1 is perfectly anisotropic (white matter). FA is sensitive to changes in white matter, but not specific at determining what aspects in microstructure result in these changes. Mean diffusivity (MD) is the mean of the eigenvalues (Equation 4.10), a measure of the local magnitude of diffusion independent of its direction. The eigenvector that corresponds to the highest eigenvalue indicates the major fibre orientation and called the principal eigenvector or principal diffusion direction (PDD) (Mori and Zhang, 2006). MD and FA is valuable to studies as indicators to neurological diseases, such as schizophrenia (Clark et al., 2011). Figure 4.3 shows examples of different maps.

$$\text{Degree of anisotropy} = (\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2 \quad (4.8)$$

$$FA = \sqrt{\frac{1}{2} \left(\frac{\sqrt{\text{Degree of anisotropy}}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \right)} \quad (4.9)$$

$$MD = \frac{D_{xx} + D_{yy} + D_{zz}}{3} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \quad (4.10)$$

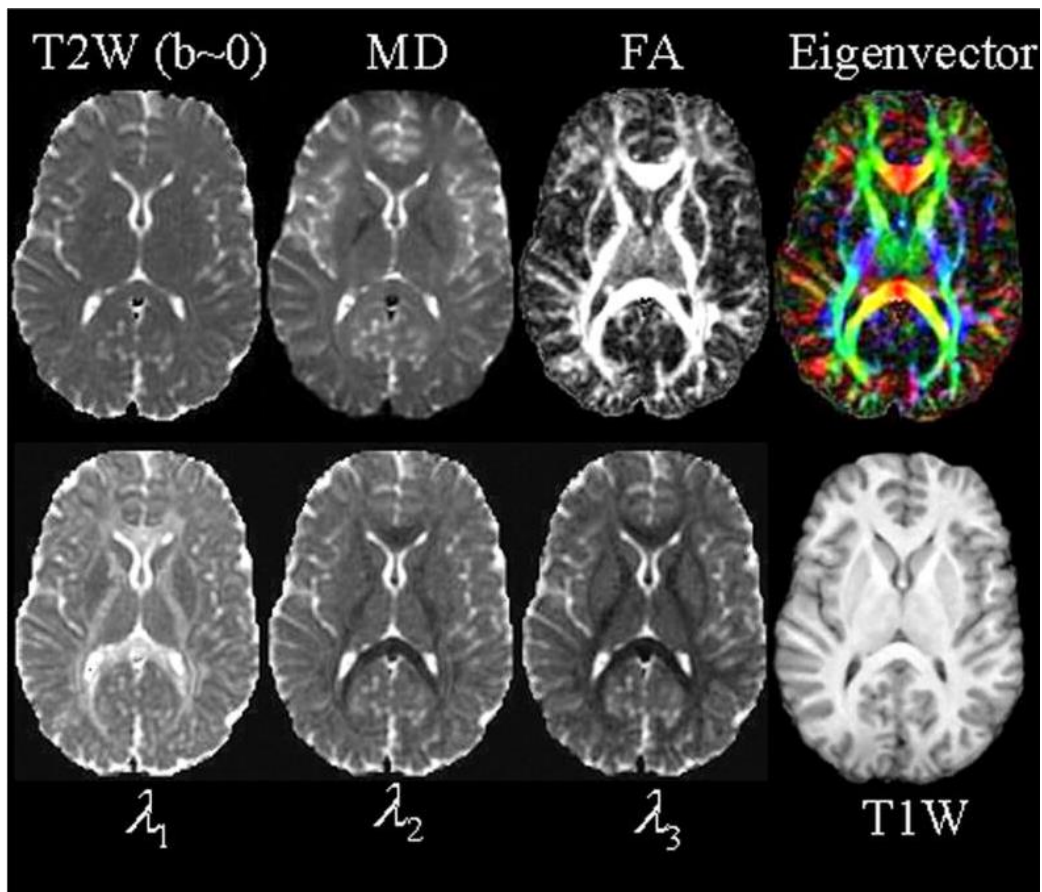


Figure 4.3. Comparison between T2-Weighted, Mean Diffusivity (MD), Fractional Anisotropy (FA), Eigenvector, three Eigenvalues (λ_1 , λ_2 and λ_3), and T1-weighted image. The colours in Eigenvector- red refers to right-left, green to anterior-posterior and blue is superior-inferior fibre orientations. Taken from (Alexander et al., 2007).

4.4 Tractography

Tractography is the process of inferring trajectories of white matter fibres from diffusion MRI data. Tractography methods rely on estimating local fibre orientations at each voxel and using these orientations to construct white matter tracts between brain regions. Diffusion tensor imaging assumes a single axon orientation per voxel, hence useful to white matter tracts that are coherently orientated (Basser et al., 2000), but multiple fibre orientations exist in the human brain. Multiple models have been devised to compensate for crossing fibres, such as the 'ball and stick' model (Behrens et al., 2007). The sticks model anisotropic diffusion to capture fibre orientations, and the ball

models isotropic diffusion attempting to capture diffusion processes that do not induce orientational contrast. Many studies have come about in the light of resolving crossing fibres issues that we will not go into details in this chapter (Descoteaux et al., 2006; Sotiropoulos et al., 2008; Sotiropoulos et al., 2013a; Sotiropoulos et al., 2013b; Tournier et al., 2004; Tournier et al., 2007; Wedeen et al., 2005).

4.4.1 Deterministic Tractography

Deterministic streamline tractography is the process of reconstructing trajectories by integrating across an orientation field. Streamline tractography assumes that white matter pathways favour aligning along preferred diffusion orientation. Commencing at a seed point we compute the principal diffusion orientation at each voxel and following this principal orientation step-by-step joining up the dots (Basser et al., 2000). Mathematically, deterministic streamline tractography can be described as a differential equation (Equation 4.11) (Fox et al., 2010).

$$\frac{d\mathbf{r}(s)}{ds} = V_1(\mathbf{r}(s)), \mathbf{r}(0) = \mathbf{r}_0 \quad (4.11)$$

This equation identifies the rate of change in position along the streamline, $\mathbf{r}$ with the local diffusion orientation (here the tensor PDD). Therefore, integrating this equation allows us to reconstruct the entire trajectory $\mathbf{r}(s)$ given a starting (or seed) point $\mathbf{r}_0$. A visual representation of streamline tractography is show below in Figure 4.4. Deterministic tractography has been used to delineate major white matter bundles in-vivo consistent with white

matter dissection studies (Catani et al., 2002). However, it can be error prone as a result of noise or inadequate modelling of fibre orientations (Johansen-Berg and Behrens, 2009a).

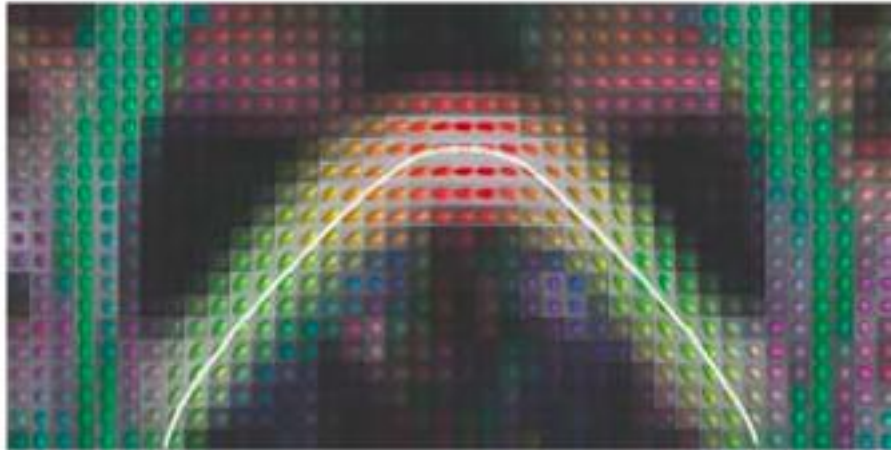


Figure 4.4. Deterministic streamline tractography representation. Fitting a tensor model to determine principal diffusion directions (PDD) at each voxel to form a trajectory and infer diffusion of least hindrance. Taken from (Johansen-Berg and Behrens, 2009a).

4.4.2 Probabilistic Tractography

Deterministic tractography relies on the estimation of local fibre orientation, which can be noisy, and excludes the uncertainty associated with each step that propagates through the tractography. Methods for estimating the uncertainty on the fibre orientation have been proposed (Behrens et al., 2003b). These form the basis of probabilistic tractography. Probabilistic tractography is a two-step process that entails estimating the principal fibre orientation and its uncertainty for each voxel to construct a path probability map of the trajectories.

During deterministic tractography we appoint two main heuristic stopping criteria, FA threshold or curvature threshold. This no longer holds in

probabilistic tractography as we account for the uncertainty associated at each voxel, hence there is no need for the streamline tractography stopping criteria and we can pass through isotropic diffusion, and paths of high uncertainty. In addition to this, probabilistic tractography reduces false-negatives.

Probabilistic tractography has many applications. It has the potential to parcellate subcortical structures into their anatomical subnuclei, using distinct anatomical connectivity to subcortical regions, such as the connectivity-based parcellation of the human thalamus as shown in Figure 4.5 (Behrens et al., 2003a), and the amygdala covered in Chapter 9. Tractography has also promising functions in surgery, including tract dissections and surgical decisions, comparison of structure-function connectomics (Chapter 6).

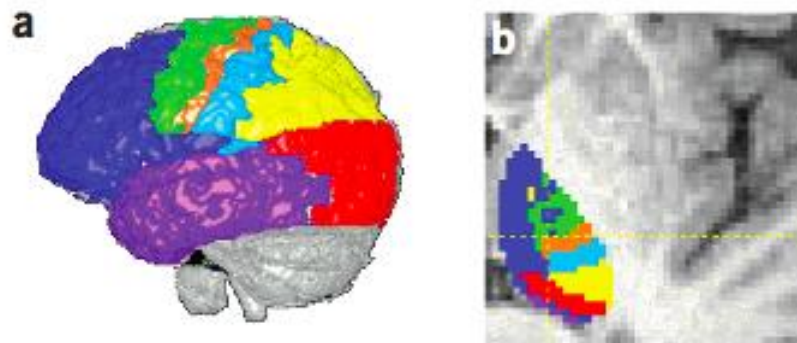


Figure 4.5. Application of probabilistic tractography for anatomical connectivity-based parcellation of the thalamus. Based on the unique projections between the thalamus (seed) and cortical surface (target), probabilistic tractography was applied between seed and target voxels to segment the thalamus into its distinct cytoarchitectural thalamus subnuclei (a) division of the cortex based on highest probable connections to thalamus subnuclei (b) segregation of thalamus voxels via probabilistic tractography. Taken from (Behrens et al., 2003a).

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Chapter 5

Functional MRI

5.1 Introduction

Functional MRI (fMRI) is a neuroimaging technique that provides insight into the functional organisation of the human brain (Figure 5.1). A change in neural activity results in blood flow changes to regions of the brain, and fMRI aims to detect these areas via the Blood Oxygen Level Dependent (BOLD) effect (Heeger and Ress, 2002). Red blood cells contain haemoglobin that carries oxygen. In its oxygenated form, called oxyhaemoglobin it is a diamagnetic, whereas in the presence of no oxygen, it is called deoxyhaemoglobin, a paramagnetic molecule. Paramagnetic molecules affect magnetic properties of spins and due to this causes local magnetic field inhomogeneity and dephasing of the local net magnetisation (Clare, 1997). This results in a decrease in MR signal due to increased T_2^* decay. Therefore, voxels with increased deoxyhaemoglobin appear darker than those with oxyhaemoglobin on a T_2^* weighted image (Heeger and Ress, 2002).

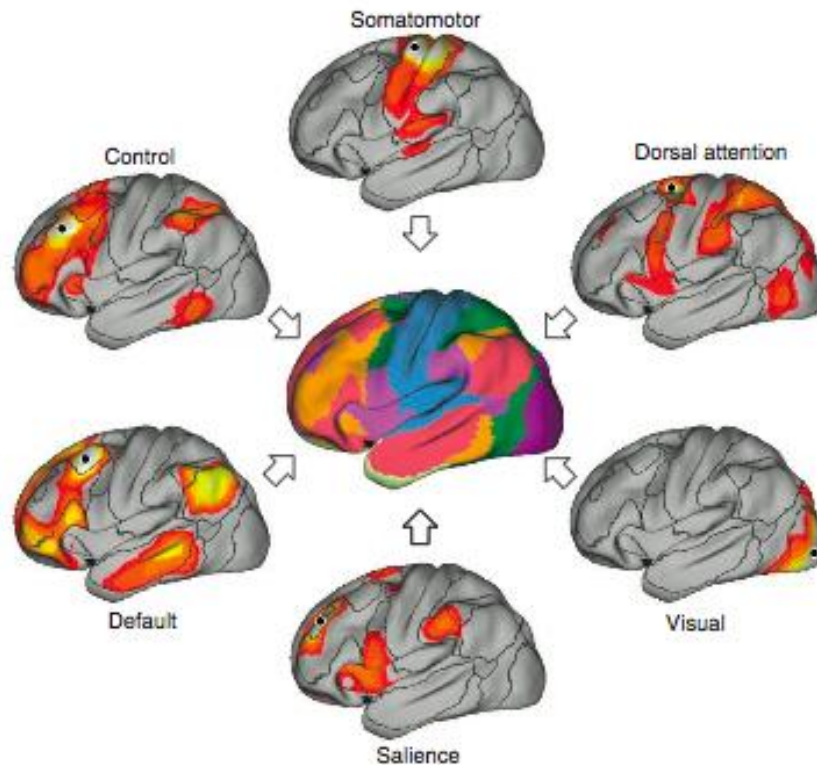


Figure 5.1. Example of cerebral cortex functional connectivity networks. Mapped from a single seed voxel (black dot) along the cortical surface, ranging from default mode network (DMN) to visual network, are examples of functional connectivity networks. The central image refers to the estimated predictions of the networks onto one surface using a best-fit approach. Taken from (Buckner et al., 2013).

fMRI is an indirect measure of how our brain's blood oxygenation level changes over time to neural activity, described as a smooth Haemodynamic Response Function (HRF) (Huettel et al., 2009). The response is called BOLD signal, illustrated in Figure 5.2 (Ogawa and Lee, 1990; Ogawa et al., 1990; Ogawa et al., 1992; Ogawa et al., 1993a; Ogawa et al., 1993b). An increase in neural activity, increases deoxyhaemoglobin levels and metabolic activity, which all require energy in the form of Adenosine Triphosphate (ATP), witnessed in some cases as a small initial dip in the BOLD signal that occurs two seconds after the detection of neural activity changes (Hu and Yacoub, 2012). Subsequent to this, more energy is needed to cope with the increased

demand and thus local cerebral blood flow is greater to supply more oxygen. However over time, there is an overcompensation of oxyhaemoglobin reaching the local region than oxygen consumed, hence causes a positive increase in response signal six seconds after neural activity. Following this, the HRF signal falls below baseline with a small undershoot due to metabolic activity, blood flow imbalance to that region and slow recovery of cerebral blood causing an increase in deoxyhaemoglobin levels (Clare, 1997), before returning back to baseline BOLD response.

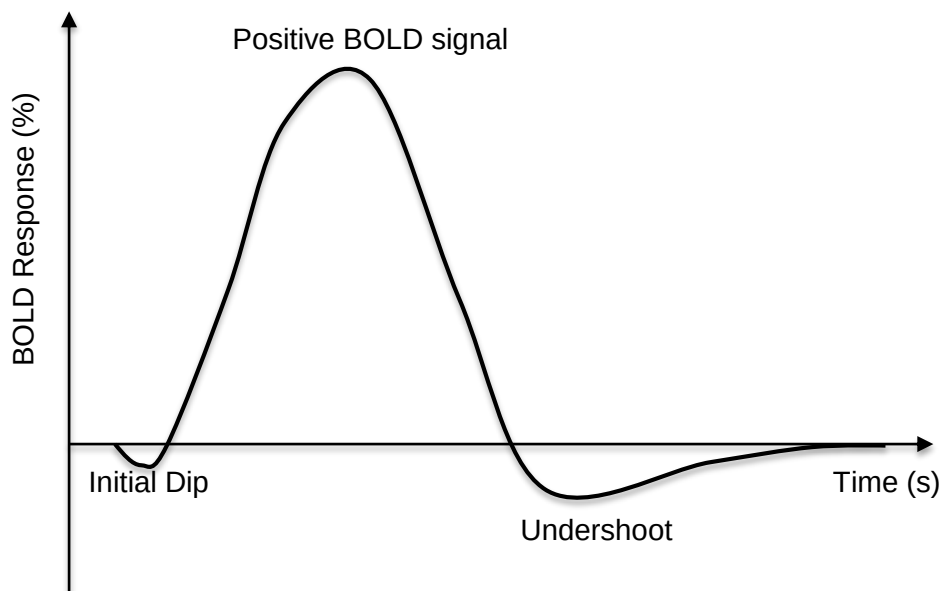


Figure 5.2. Schematic of Haemodynamic Response Function (HRF) to stimulus. An increase in neural activity increases deoxyhaemoglobin levels resulting in a small initial dip. Over time, local cerebral blood flow increases to provide more oxygen to the local brain region increasing BOLD signal response, though eventually the signal decreases below baseline in an undershoot before returning back to baseline levels.

Since the development of Seiji Ogawa findings researchers have been devising fMRI investigations to determine brain activation to different conditions or stimuli. This entails the investigation of fMRI experimental design, acquisition, and statistical data analysis.

5.2 Acquisitions and Experimental Design

In Chapter 3 we covered MR principles and the different areas essential in MRI systems. Gradient echo (GE) EPI is popular in fMRI due to its high image quality, speed and sensitivity to BOLD effect. By altering imaging parameters (flip angle, TR, TE etc.) we can achieve tissue contrasts to distinguish between white matter, grey matter and CSF, and increases signal to noise ratio (SNR) whilst reducing artefacts.

Temporal resolution (sampling rate) is defined as the smallest two time points in an fMRI time series we can differentiate between. It is dependent on experimental design and estimation of the HRF response that in turn limit such resolution. Temporal resolution is also dependent on TR and ideally should be long enough to give time for MR signal recovery before applying the next pulse, typically in the range of 1-1.5 seconds. A shorter TR is also associated with fewer slice acquired and hence less brain coverage (Amaro and Barker, 2006).

Spatial resolution is the ability to distinguish between two spatial locations of independent units, dependent on voxel size and slice thickness, limited by MRI hardware. Smaller voxels have less cerebral blood flow, fewer spins and hence lower signal, in addition to requiring longer scanning time, resulting in increased noise as subjects become more restless and move within the scanner. Whereas, too large a voxels will decrease CNR due to partial volume effects (Clare, 1997). Furthermore, as slice thickness increases there is an

increase in signal, but greater signal dephasing (higher dropout) and an increase in physiological noise, resulting in a decrease in signal.

Physiological noise is an internal noise, caused by cardiac pulses, respiratory rate, or movement of subjects during scanning (Le and Hu, 1996). Physiological monitoring during scanning is advantageous in determining variations in fMRI data that can be corrected for via analysis approaches. Increasing magnetic field strength increases SNR, but is limited by physiological noise. This results in greater dephasing of local magnetisation due to susceptibility differences, especially prominent in some regions of the head, such as the sinuses (Clare, 1997).

fMRI is classified into two types; task fMRI or rest state fMRI (R-fMRI). During task fMRI, a subject is presented with a stimulus or asked to perform an action repeatedly to achieve high enough CNR to identify areas of activation and their functional subregions in the human brain. A block or event-related task design can be applied to achieve task fMRI. Firstly, block design entails a series of on/off or stimulus/rest alternating pattern at discrete regular epochs, which are each long enough to capture the HRF response (greater than eight seconds) (Friston et al., 1999). Therefore, a region of the brain will become activated or deactivated in correspondence to the task set in hand, and the signal retrieved is then compared to other stimulus conditions. On the other hand, an event-related task is made up of stimulus presented for short periods of time that are measured separately. This thesis focuses entirely on R-fMRI, and task fMRI is not discussed further.

R-fMRI aims to investigate functional interactions between regions, whilst a subject is not engaged in any explicit task. Usually the subject remains still with either their eyes closed, open or fixated upon a point throughout all scanning sessions. At rest state (Raichle, 2011), 0.1Hz low frequency fluctuations were found present in BOLD R-fMRI signal time series, which correlated with long-range brain regions (Biswal et al., 1995). An example is the default mode network (DMN), and its function is still under much debate (Buckner et al., 2008).

5.3 Analysis of fMRI Data

The raw data obtained from the fMRI study must be pre-processed to minimise noise and artefacts, and a favourable approach is FMRIB Software Library (FSL) (Jenkinson et al., 2012) and FreeSurfer (Fischl, 2012). Thereafter, statistical methods are employed to perform analysis and determine active voxels. fMRI has shown major clinical and translation applications (Matthews et al., 2006), from neurosurgical planning to characterisation of neurological diseases.

5.3.1 Pre-processing

Motion Correction

Subjects either physically or involuntarily (cardiac-respiratory cycle) move within a scanner. As a result, voxels contain data from different brain locations, and all sources of error must be rectified. Analysis tools, such as MCFLIRT can correct for motion (Jenkinson et al., 2002). This entails the use

of both registration and optimisation to robustly align volumes to minimise error based on transformations, such as rotation and translation.

Spatial Smoothing

Spatial smoothing improves SNR. This is accomplished by blurring the image via local averaging of neighbouring voxels to reduce noise at the expense of reducing resolution. A Gaussian kernel of Full Width Half Maximum (FWHM), which determines the degree of smoothing, is convolved with the fMRI image via Fourier Transform (FT). The kernel is arbitrary, but it is ideally smaller than the areas of activation of interest as to not reduce the signal as well as noise.

Temporal Smoothing

Another approach of increasing SNR is temporal smoothing. A low-pass and/or high-pass filter is applied to each voxel's time series in the Fourier domain. The latter aims to reduce low frequency noises occurring due to drifts from physiological effects and scanner-related drifts. As the HRF response is smooth, employing a low-pass filter eliminates high frequency components from the fMRI time series voxel, hence increasing SNR. On the other hand, a low-pass filter can be applied in a similar way to the technique used in spatial smoothing, using the Gaussian kernel, though this approach can result in false positives (Jezzard et al., 2003).

Image Registration

Each subject is mapped to its own anatomical space, though to perform analysis they must all be aligned and registered to one common space, such

as Montreal Neurological Institute (MNI) 152 standard space. FMRIB's Linear Image Registration Tool (FLIRT) uses robust registration algorithms to register each subject's fMRI image onto one common space (Jenkinson and Smith, 2001; Jenkinson et al., 2002).

5.3.2 Statistical Analysis

Pre-processing of R-fMRI and task based fMRI is similar though statistical analysis varies considerably. At task fMRI, analysis aims to determine which voxels show significant activation to a stimulus via t-test, correlation analysis or modelling thus achieving activation maps. These voxels are analysed independently, via univariate or all together via multivariate analysis. Multivariate analysis can be classified into model-based or model-free methods (Smith, 2004). The most common model-based approach is General Linear Model (GLM).

On the other hand, analysis of R-fMRI data can be dealt with via independent component analysis (ICA) or seed-based analysis (Cole et al., 2010). ICA enables resting state network detection, such the DMN. ICA requires less prior assumptions though the number of components are arbitrary (Lee et al., 2013), with the aim of separating out the fMRI multivariate time series signal into n independent components (Beckmann et al., 2005; Damoiseaux et al., 2006). This is valuable in eliminating noise from the signal arising from scanner-related issues and physiological noise, though it requires manual selection. FIX (FMRIB's ICA-based Xnoiseifier) is a tool that enables the auto-classification of ICA components into 'good' versus 'bad' components for

potential removal of these bad components from four-dimensional fMRI data (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014).

Another approach of R-fMRI analysis is using regions of interest (ROIs) or masks that are constructed either manually or through automated parcellation techniques to extract the individual fMRI voxels time series of the ROIs. This method requires ROI selection beforehand (Lee et al., 2013). Following this, via a voxel-based approach the time series of each voxel are correlated with other voxels in the ROI and the remainder of the brain. This forms the basis of seed based correlation analysis (Mars et al., 2011; O'Reilly et al., 2010). A recent study shows that ICA and seed based analysis provide similar results in healthy participants (Rosazza et al., 2012), and for the purpose of this thesis we focus on seed based analysis for parcellation of the amygdala (Chapter 8).

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Chapter 6

Structure-Function Connectomics

6.1 Introduction

Networks are ubiquitous, from the World Wide Web and Transport System to Neurobiology and Social Networks. Complex networks are often formalised and analysed using tools from Graph Theory. A graph is a set of vertices or nodes, N connected by edges, E (Figure 6.1) (Bullmore and Sporns, 2009), where edges can be directed or undirected, weighted or unweighted (Bassett and Bullmore, 2009). Networks are a powerful way of understanding the structure and functions of complex systems, such as the human brain. In this chapter, we explore structural and functional brain networks at a macroscopic level (or macro-connectome), as measured through in vivo MRI.

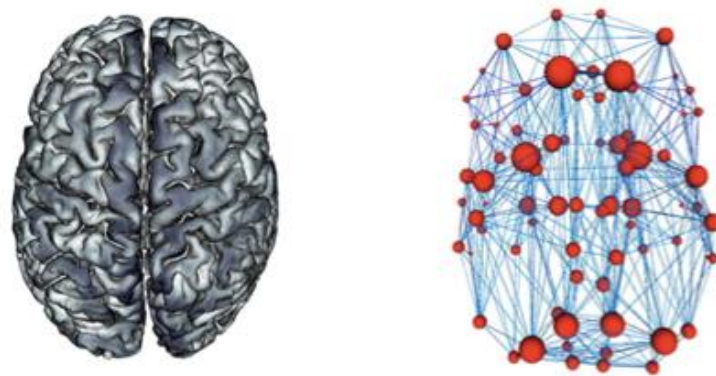


Figure 6.1. Example of a static complex brain network. Edges (blue) connect nodes (red). Edges can be synapses (micro-connectome) or fibre tracts (macro-connectome). Figure taken from (van den Heuvel and Sporns, 2011).

6.2 Structural Connectome

The structural connectome is the set of physical connections between distinct brain regions. Dissections, tracer studies and neuroimaging allow us to investigate these connections, however the current gold standard in measuring structural connections relies on axonal tracer studies. Unfortunately, these techniques are invasive and not feasible in human brains. Diffusion imaging cannot differentiate between afferent and efferent projections, and detect intrinsic projections (Park and Friston, 2013), but is a non-invasive and in-vivo technique (Chapter 4).

We constructed an intra-hemispheric structural brain network. Diffusion-weighted (DWI) and T1-weighted images were obtained from five healthy subjects (aged 20-35), selected from the FMRIB database and acquired on a 3T Siemens Trio scanner. Diffusion-weighted images (DWI) were acquired using Spin-Echo (SE) EPI sequence (TR=10s, TE=94ms, voxel size= 2mm isotropic, b-value=1000 mm^2 along 60 diffusion gradient directions). Diffusion data were pre-processed using FSL (Jenkinson et al., 2012; Smith et al., 2004). This included EPI distortion correction via TOPUP algorithm (Andersson et al., 2003; Smith et al., 2004), eddy current and motion correction using EDDY (Andersson et al., 2012), and MNI 152 2mm space transformation via FMRIB's Nonlinear Image Registration, FNIRT (Andersson et al., 2007).

For each subject, Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques, BEDPOSTX was applied to estimate brain voxel

crossing fibres with a maximum of two fibre orientations per voxel (Behrens et al., 2003a; Behrens et al., 2003b; Behrens et al., 2007). We performed probabilistic tractography, probtrackx, seeding from the left hemisphere with 150 samples per voxel to estimate the probability of connectivity between grey matter voxels (Behrens et al., 2003a; Behrens et al., 2003b; Jenkinson et al., 2012). A termination mask was applied to stop tracts passing through the right hemisphere. The average connectivity probability per voxel was computed across the five subjects, and projected onto a surface model of the left cortical hemisphere with 10,001 vertices, resulting in a 10,001 square non-symmetric, sparse, and weighted structural matrix representing the connectivity density between vertices. Furthermore, non-zero structural connectivity matrix elements were log transformed.

6.3 Functional Connectome

Resting-state functional connectivity indirectly measures brain connectivity by determining statistical dependence between nodes through correlation, spectral coherence, or covariance (Friston, 2011). At rest, 0.06-0.1Hz low frequency fluctuations in BOLD signal time series were found to be consistent across subjects, correlating with long-range brain connections (Damoiseaux et al., 2006; De Luca et al., 2006; Yeo et al., 2011).

We constructed the functional connectome of the left human brain at rest. Twelve subjects, aged between 20 and 35 were scanned for 6 minutes using a Gradient Echo (GE) EPI sequence via a 3T Siemens Trio scanner (TR= 2s, TE = 28 ms, flip angle= 89 deg, field of view=224mm, voxel size =2mm

isotropic). Pre-processing included motion correction (Jenkinson et al., 2002), brain extraction (Smith, 2002), 6mm full-width at half- maximum (FWHM) spatial smoothing. R-fMRI data was registered to MNI standard space FMRIB's Nonlinear Image Registration, FNIRT (Andersson et al., 2007), before being re-sampled into the 10,001 surface vertices. For each vertex we computed the correlation between R-fMRI time series and averaged the results across the twelve subjects to construct a full symmetric, non-sparse and weighted functional connectivity matrix between the vertices.

6.4 Correlating Structure and Function

It has been suggested that the functional organisation of the human brain may be shaped and constrained by its underlying physical wiring (Honey et al., 2009; Honey et al., 2010). This implies that the structural and functional connectome should show a high degree of correlation. We set to test this hypothesis using the data described above.

Structural and functional connectivity matrices were constructed using the data and methods described in the two previous sections. Each matrix element corresponds to the connectivity between one vertex and another. For analysis, all data was normalised (ranging from 0 to 1), and thresholded. Equation 6.1 illustrates the normalisation process of each row for both structural and functional connectivity matrix, where k is an element of a row, and K_{min} and K_{max} refers to the minimum and maximum value of row, respectively.

$$Normalisation(k) = \frac{k - K_{min}}{K_{max} - K_{min}} \quad (6.1)$$

Figure 6.2 illustrates the structural and functional connectivity distributions of a vertex, situated at the left lateral prefrontal cortex, using different thresholds. A 25% threshold was applied to each matrix row to decrease noise. To further increase SNR and reduce data complexity, we clustered the vertices into a set of 100 cortical regions of interests (ROIs). These ROIs were estimated using random seeds positioned across the cortex and clustered via voronoi approach, based on their geodesic distance on the cortical surface (Figure 6.3). Voronoi clusters vertices based on their closeness to other vertices on a region- a random number of centre vertices, N were selected over the left hemisphere cortical surface and other vertices were assigned to the nearest centre based on their distance to each other, hence producing a parcellation into similar-sized parcels. We further computed the R-fMRI and DTI mean connectivity and euclidean distance between each pair of ROIs (100 x 100 matrix size).

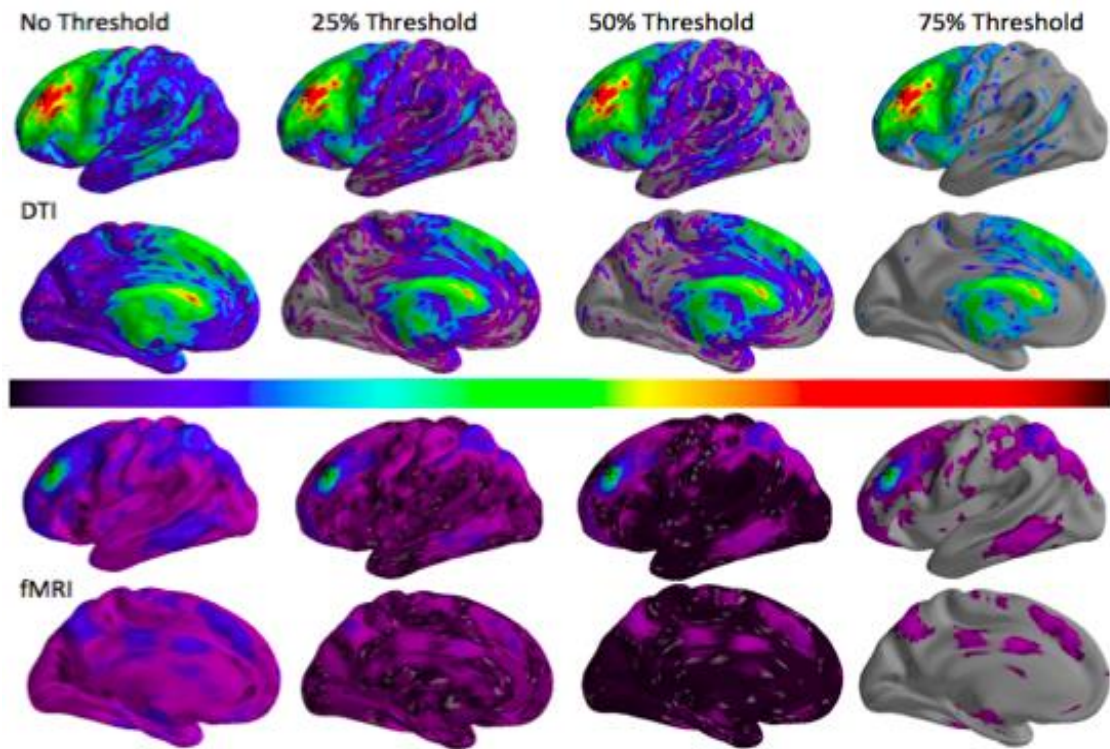


Figure 6.2. Thresholding of the human connectome. Structural and functional connectivity of a vertex positioned on the left lateral prefrontal cortex at different thresholds. 25% threshold provides the most optimal SNR.

To compare the structural and functional connectomes, we calculated for pair of ROIs, the Pearson correlation coefficient between their normalised structural and functional connectivity to the remaining 98

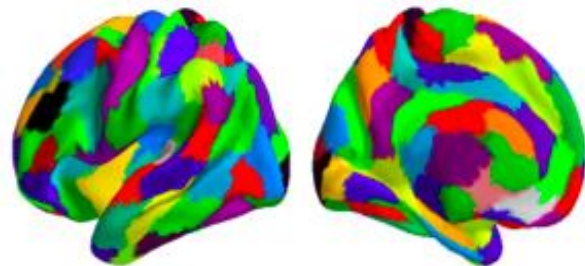


Figure 6.3. 100 regions of interest (ROIs) via Voronoi-type clustering on the left-brain cortical hemisphere.

regions. The results can be visualised as a correlation matrix (Figure 6.4(b)). We observe strong diagonal-dominance, indicating that the strongest correlation between an ROI's functional connectome is its own structural connectome. However, this analysis may also be confounded by distance, as

both structural and functional connectomes show a high degree of localisation, i.e. strong local connections and sparse long-range connection. The following section aims to examine this distance dependence more closely.

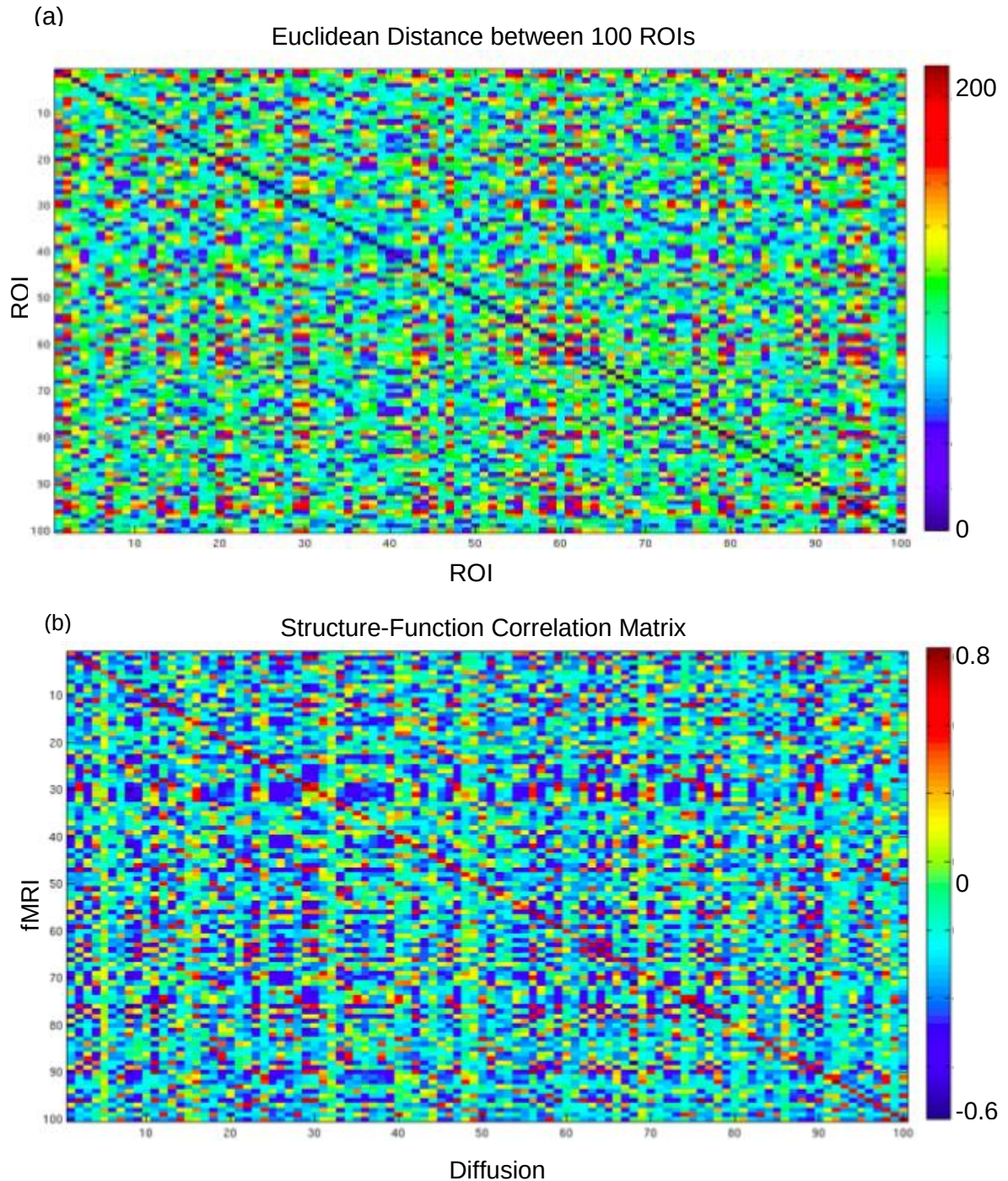


Figure 6.4. ROI euclidean distance and structural-functional correlation matrices (a) Euclidean distance between 100 ROIs following parcellation using voronoi-type clustering. (b) Structural-functional correlation matrices. Diagonal dominance shows ROIs most correlated with themselves than with other ROI though other high correlating ROIs are evident, with maximum correlation being 0.8 and minimum -0.6.

6.5 Distance Dependence

We empirically observed that the average connectivity between regions is approximately inversely proportional to fibre distance, $R=0.46$ for structural and $R=0.25$ for functional connectivity (Figure 6.5). Honey et al. (2009) witnessed similar findings ($R=0.67$ for R-fMRI) (Honey et al., 2009).

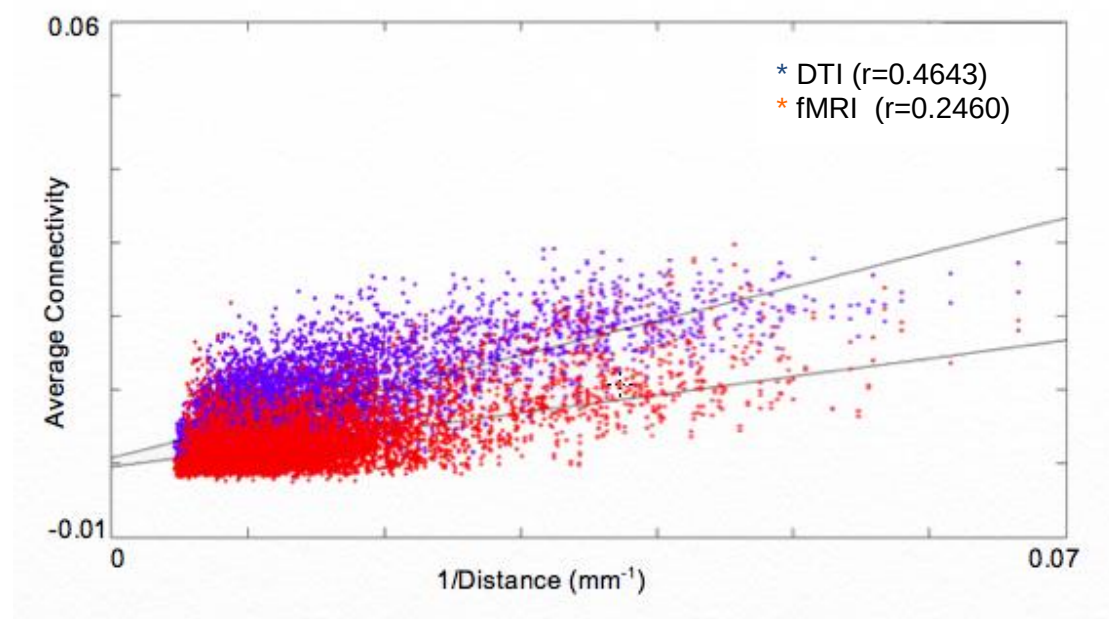


Figure 6.5. R-fMRI and DTI average connectivity inversely proportional to distance. $R=0.46$ for diffusion and $R=0.25$ for function. This further implies that the correlation between an ROI's functional connectivity is its own structural connectome. Regressing out the distance effect using a Rational Quadratic Model (RQM) boosts long-range connections for exploring their relationship.

In order to focus on large-scale connectivity, we fitted a Rational Quadratic Model (RQM) to both structural and functional connectivity ROIs, as shown in Figure 6.6(a) (Bellec et al., 2006). An RQM model is a parametric spatial correlogram that models the correlation between two regions based on their spatial euclidean distance from each other. This allows the identification of local versus large-range connections (Figure 6.6(a)), based on a threshold

critical distance h_{∞}^{ε} of 1.5cm with a tolerance, ε of 0.01. Figure 6.6a formulas are empirical estimates of the correlogram over distance, h . The RQM is fitted to each row of the diffusion and resting-state fMRI matrix, where $\theta = (\theta_1, \theta_2, \theta_3)$ is a non-negative parameter vector satisfying $\theta_1 + \theta_2\theta_3 < 1$ defining a class of valid correlograms. RQM starts with a maximum connectivity, ρ_{0+} that defines local connections, and decreases asymptotically to a correlation of ρ_{∞} of long-range correlations. Figure 6.6(b) shows the normalised diffusion connectivity between one ROI to all its other ROIs with a fitted RQM. Regressing out the distance effect resulted in weaker diagonal-dominance (Figure 6.7). Figure 6.8 (a) and (b) illustrates the diagonal elements of the correlation matrix mapped onto the cortical cortex, before and after regression. Overall, the pattern of correlation between structural and functional connectomes appears to be preserved after regressing out the distance dependence. However, there are also some notable differences, with some regions where the correlation increases and some others where it decreases, such as the dorsal posterior cingulate cortex and middle temporal gyrus, respectively. These correlations is strongly suggested for efficient brain wiring whilst minimising wiring cost between region's connectivity patterns (Bullmore and Sporns, 2012).

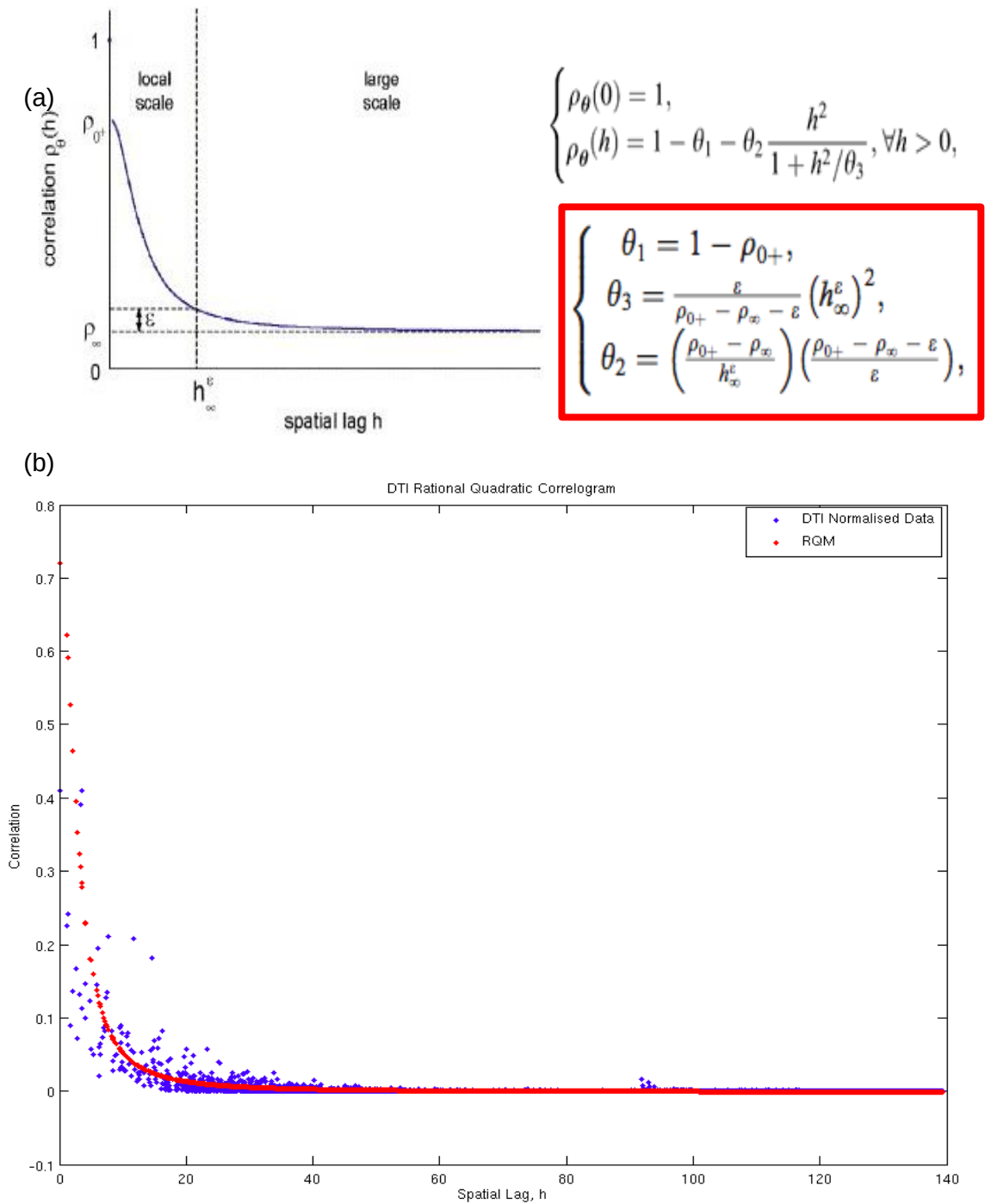


Figure 6.6. Rational Quadratic Correlogram (RQM) (a) RQM regresses out distance effect between ROIs modelled by a series of equations (red box). The model attempts to eliminate local scale connectivity for exploration of large range connections. Taken from (Bellec et al., 2006). (b) Implementation of RQM to diffusion data resulting in residual data points analogous to long-range cortical connections.

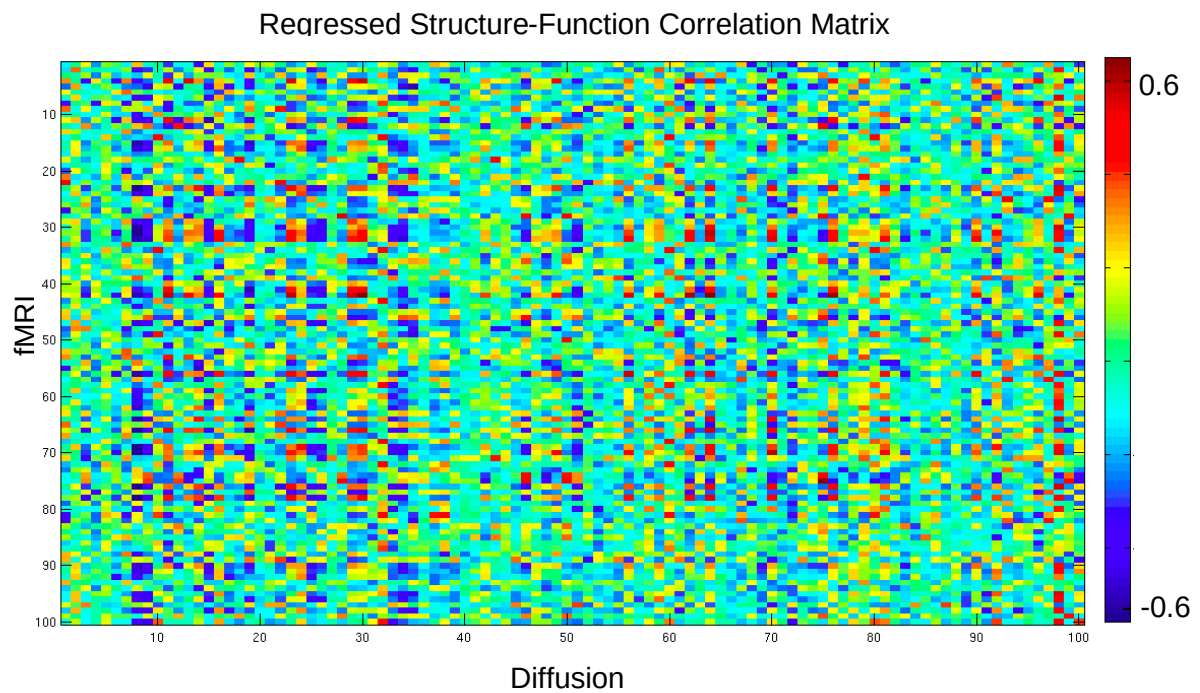


Figure 6.7. Structural-functional correlation matrices following euclidean regression. Matrix highlights lack of diagonal dominance in comparison to before regression in Figure 6.4(b). Maximum correlation being 0.6 and minimum -0.6.

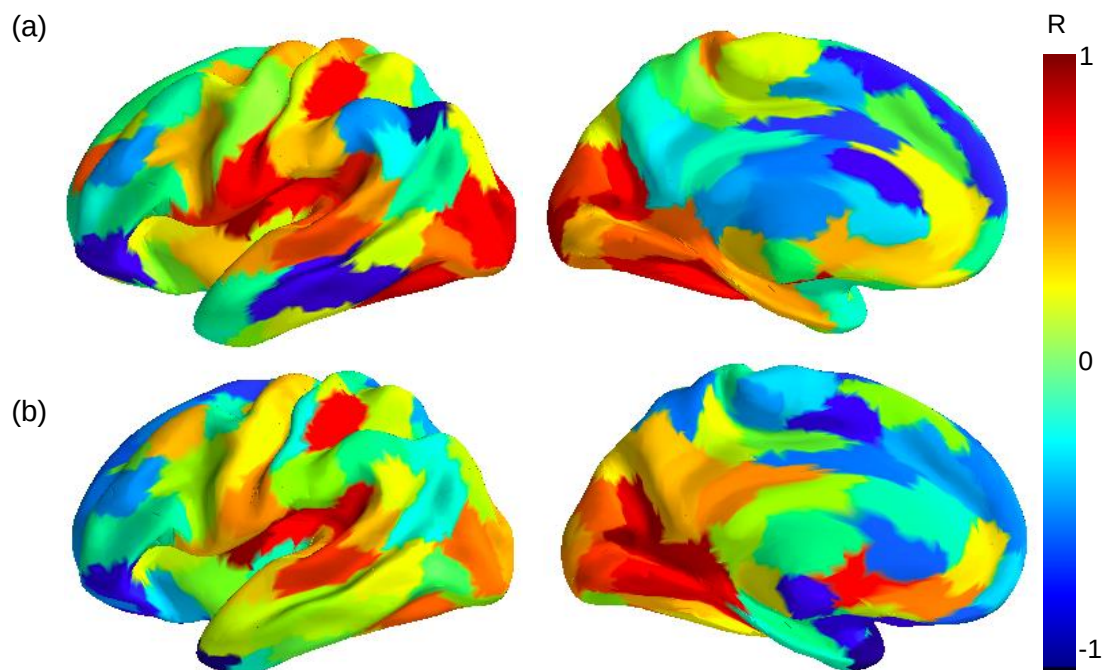


Figure 6.8. Diagonal elements of the correlation matrix projected as a map of the cortex (a) Before distance regression (b) After regression. Some regions show an increase in correlation after regression and some decreasing, such as the dorsal posterior cingulate cortex and middle temporal gyrus, respectively. These correlations are likely for efficient brain wiring while minimising wiring cost between region's connectivity patterns (Bullmore and Sporns, 2012)

6.6 Discussion

Brain networks enable us to explore the structure and function of the human connectome (Sporns et al., 2005; Zalesky et al., 2010). Diffusion imaging is a non-invasive MRI technique that allows us to infer anatomical white matter organisation by following its preferred direction of diffusion. On the contrary, resting-state fMRI enables the measurement of Blood Oxygenation Level Dependent (BOLD) signal response at rest, inferring functional connections between brain regions. Functional connectivity is thought to be a reflection of the underlying structural connectivity yet their relationship is unclear (Cabral et al., 2012; Honey et al., 2009). We studied the functional and structural intra-hemispheric connectome, and highlight similarities between both modalities.

10,001 vertices were positioned along the left-brain's cortical surface and their average structural (subject=5) and functional (subject=12) connectivity matrices computed. All data was normalised and a 25% threshold applied to decrease noise. Furthermore, to increase SNR and reduce data complexity, we partitioned the 10,001 vertices into 100 cortical region of interests (ROIs), estimated using random seeds positioned along the cortex and clustered in a voronoi-type fashion, based on their euclidean distance. This form of random parcellation is not an ideal representation of the human brain, and an anatomical-based parcellation would be more advantageous. Other techniques, such as the principles of segregation and integration would be a more ideal form of defining nodes (Tononi et al., 1994), as anatomical parcellation more resembles the interactions between human brain regions.

Functional and structural connectivity relationship between pairs of ROIs was calculated using pairwise Pearson correlation coefficient. We observe the strongest correlation between an ROI's functional connectome is its own structural connectome. However, such correlations may be driven by a 'distance bias' that favours short to long connections in both datasets. Such a bias may be due to the smoothness of the data, but is also a feature of cortical anatomy evident in a recent tracer study by Markov, N et al. (2013) (Markov et al., 2013). In order to test whether our structural-functional correlation was purely driven by this distance effect, we used a Rational Quadratic model to regress-out distance from the connectivity data, effectively boosting long-range connections. We observe that, while the overall picture of structural-functional correlation remains, there are changes in correlations before and after regression, such as the dorsal posterior cingulate cortex. Such networks optimise brain connectivity, reducing cost and axonal volume whilst increasing efficiency (Behrens and Sporns, 2011; Bullmore and Sporns, 2012). As the euclidean distance does not capture cortical folding future work must be employed to remove such bias. Furthermore, repetition of these studies with more subjects and higher resolution data, for example the Human Connectome Project (HCP) (Van Essen et al., 2013) could further validate these findings.

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Chapter 7

Topographic Organisation of the Human Connectome

7.1 Introduction

The principles of segregation and integration are properties exhibited in the human brain (Tononi et al., 1994). A group of specialised neurons are organised into discrete units to interact and perform distinct behaviours (Tononi et al., 1998), but variations in connectivity patterns between spatially homogenous regions exist. We explore one type of connectional organisation, topographic connections. These patterns include gradual connectivity changes or superimposition of brain wiring patterns to perform complex behaviours (Jbabdi et al., 2013). Some researchers deem topography to be non-fundamental (Hebb, 1949), whilst others oppose this motion (Kaas, 1997).

7.2 Nature of Topography

To date, topographic connections have been ignored when exploring brain networks. Topographic connections are point-to-point mappings aimed at preserving spatial connectivity patterns between regions (Jbabdi et al., 2013; Thivierge and Marcus, 2007; Udin and Fawcett, 1988). Roger Sperry produced one of the first recorded evidence of such organised projections pioneering the theory of chemoaffinity hypothesis (Sperry, 1943). A frog's optical nerves were severed and its eyes rotated by 180 degrees. As the

frog's nerves regenerated its retinal axons grew back with the frog's field of view inverted, whilst its connectivity pattern remained the same (Udin and Fawcett, 1988). This is an example of a preserved topographic connection.

Sperry's findings are an insight into topographic organisation of the visual sensory system. Other topographic maps found in the human brain, include retinotopic (Wandell et al., 2007), somatotopic (Stippich et al., 2002), and tonotopic (Saenz and Langers, 2014). These maps are found between sensory organs and the cortex, however evidence of spatial arrangements between grey matter association regions in primate's are also present (Amaral et al., 2003; Suzuki and Amaral, 1994).

Topographic connections are detectable via electrical stimulation of sensory areas (Kaas, 1997), direct stimulation of the cerebral cortex (Metman et al., 1993), single cell recording (Hubel, 1963; Hubel and Wiesel, 1998), and neuroimaging (Engel et al., 1994). Due to the non-invasive nature of neuroimaging, functional MRI has enabled us to detect ordered projections, such as retinotopic maps in the visual cortex and other cortical areas (Arcaro et al., 2011; Saygin and Sereno, 2008). This is often accomplished via task-based fMRI, but resting-state fMRI and diffusion imaging has also been employed recently to detect topography in regions not involved in sensory roles (Cauda et al., 2011; Taren et al., 2011).

7.3 Gradient Topographic Connections

The range of topographic connections include uniform projections (Figure 7.1(a)) (Suzuki and Amaral, 1994), clusters (Figure 7.1(d)) (Schall et al., 1995) and gradients.

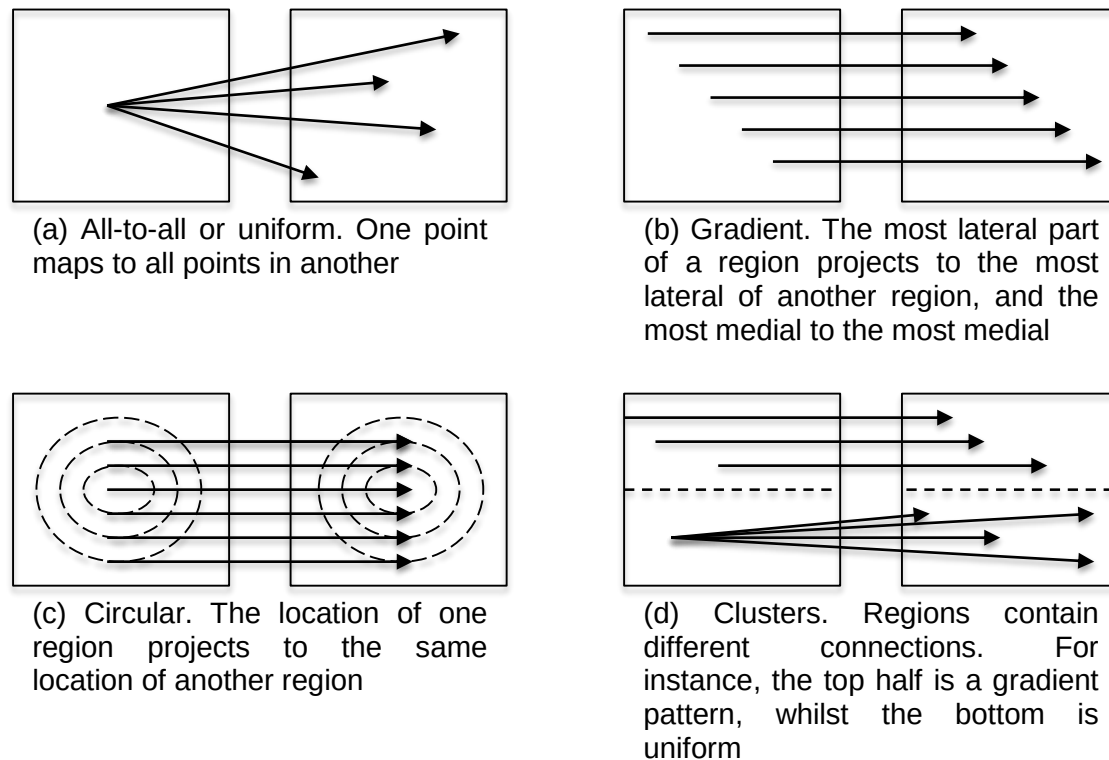


Figure 7.1. Types of topographic connections.

Researchers have shown evidence of gradient projections in macaque monkeys (Amaral et al., 2003). The connectivity pattern between a macaque's Entorhinal (E_c), Perirhinal and Parahippocampal (P_c) Cortex is a classic example (Figure 7.2). Projections from Perirhinal to E_c are all-to-all or uniform; one point in the Perirhinal projects to any point in the E_c . Whereas, projections from P_c to E_c follow a spatial mediolateral gradient topographic pattern- medial parts of P_c project mostly to medial parts of E_c and lateral parts of P_c project

mostly to lateral parts of E_c (Suzuki and Amaral, 1994). E_c comprises of two types of connections within the same target region, all-to-all and gradient connectivity. This overlap of connections may be classed as the fundamentals in performing complex calculations and transformations (Jbabdi et al., 2013).

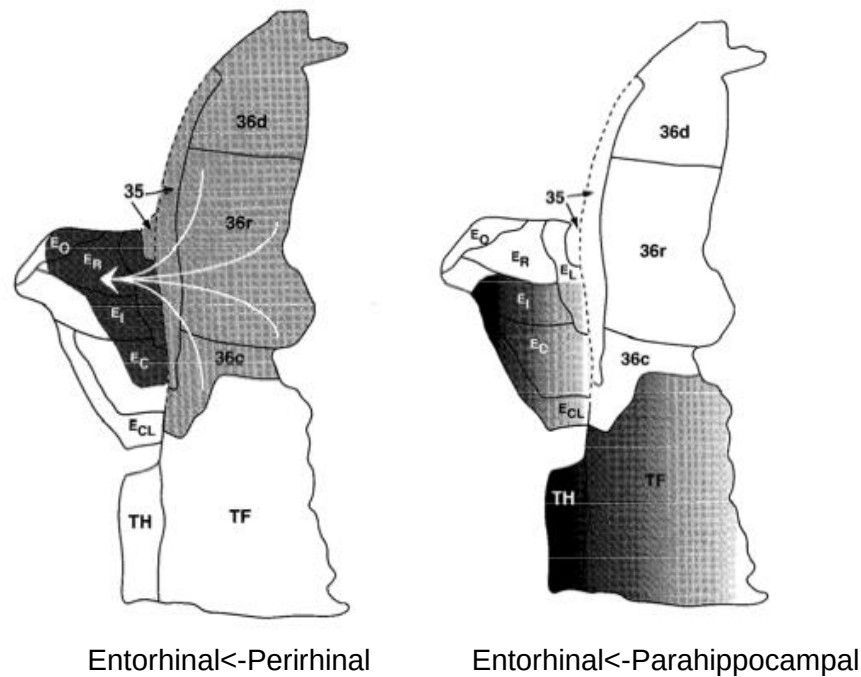


Figure 7.2. Topographic organisation between macaque monkey Entorhinal Cortex (E_c), Parahippocampal Cortex (P_c) and Perirhinal Cortex. Perirhinal to E_c projections are uniform, whilst P_c to E_c show gradient spatial pattern. This is an example of clustered topographic connections.

In this chapter, we show attempts at modelling spatial gradients of connectivity, with the aim of developing a method for detecting such gradients from in vivo connectivity data.

We modelled and simulated spatial gradients of connectivity on a spherical model of the cortex. The sphere was parcellated into N regions of interests (ROIs), using a random parcellation into contiguous areas of approximately equal size. Gradient of connectivity between any pair of ROIs was defined by

first selecting an axis tangent to the sphere on each of the ROIs, which defines the orientation of the mapping. Connectivity was mathematically defined using equations 7.1 and 7.2. Given the position of each ROI vertex, $ROI_{1(2)}$, Ax is the normalisation of each vertex with respect to its mean distance along an axis tangent. The gradient connectivity probability, P is expressed in Equation 7.2, where σ is the standard deviation of a Gaussian distribution and γ is constant parameter. Figure 7.4 is a normalised gradient connectivity simulation between two ROIs at $\sigma=0.5$.

$$Ax_{1(2)} = \frac{ROI_{1(2)} - \text{Mean}(ROI_{1(2)})}{\text{Length}(ROI_{1(2)})} \quad (7.1)$$

$$P = \gamma e^{-\frac{|Ax_1 - Ax_2|}{\sigma^2}} \quad (7.2)$$

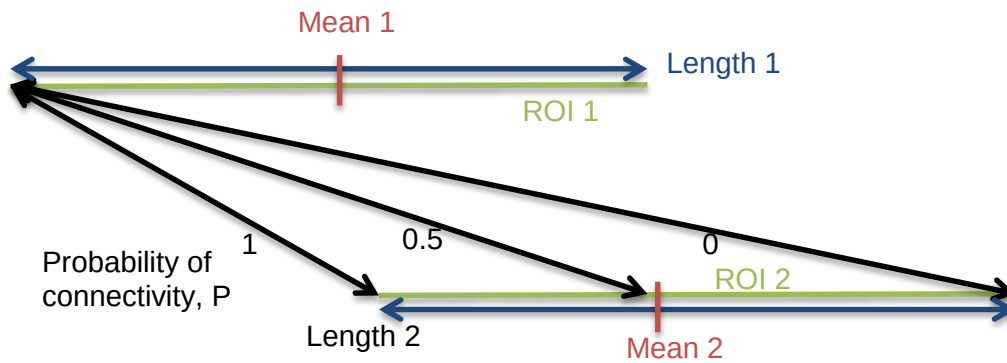


Figure 7.3. One dimensional gradient connectivity model between two ROI's. Regions closer to one another have a higher probability of being connected than those further away, normalised by the distance of the ROI.

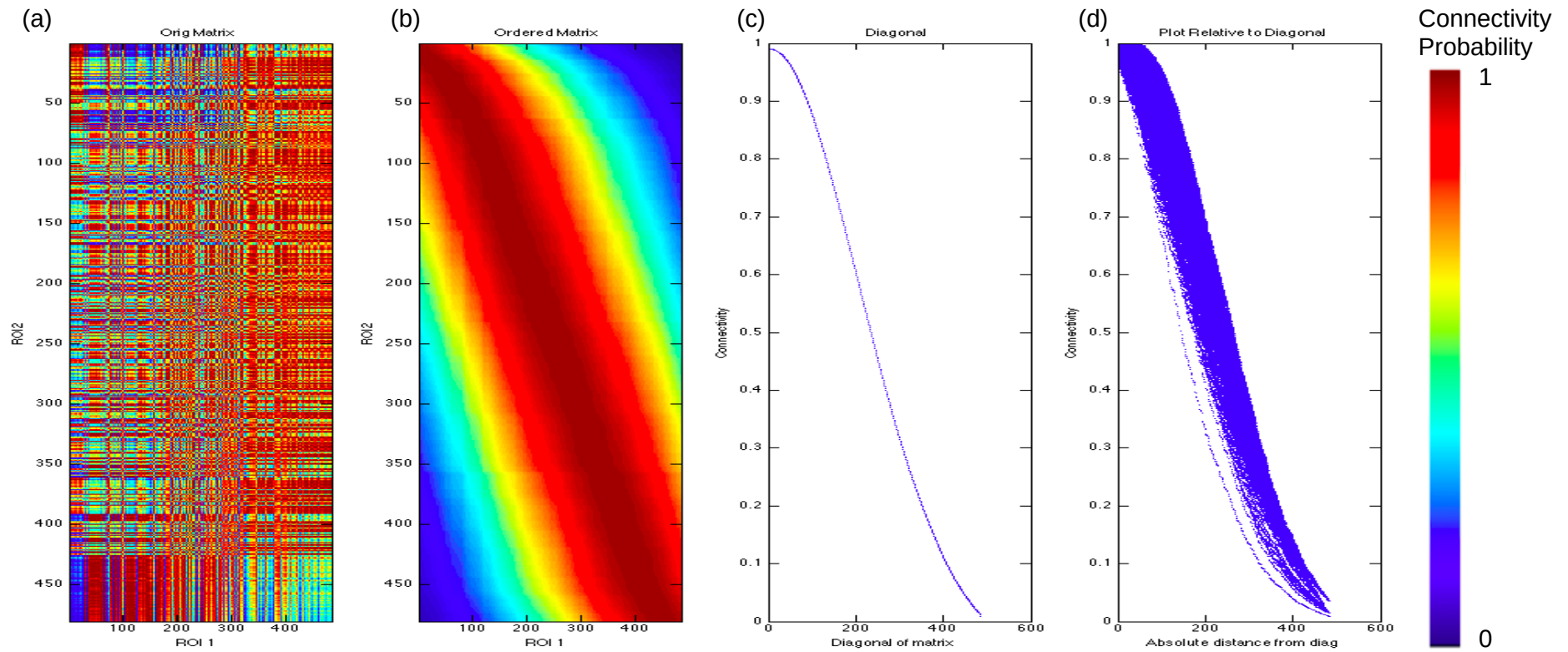


Figure 7.4. Simulation of a gradient connectivity pattern between two ROIs at $\sigma=0.5$. (a) Gradient connectivity probability between ROI 1 and ROI 2. Red refers to high connectivity probability of connectivity and blue is lower probability connectivity. (b) Re-ordering of 7.4(a) matrix computed via the cost-function (c) The diagonal of the gradient connectivity matrix, determined by taking the maximum connectivity between vertices of ROI 1 and ROI 2 (d) The distance of each element of 7.4(b) matrix relative to the diagonal based on 7.4(c).

In addition to this, topographic gradients can include different transformations such as dilation, rotation, reflection and translation. In the next section, we explore whether, given a simulated one-dimensional gradient of connectivity as described above, can we detect the angle between the two tangent vectors in the two ROIs. Two solutions were considered based on an ad-hoc cost function optimisation and spectral re-ordering.

7.3.1 Cost Function and Spectral Re-Ordering

Given a simulated connectivity matrix between two ROIs, the first approach to finding the axes of gradients in connectivity was to rotate one of the two ROIs and re-order the rows of the connectivity matrix following this rotation. If a one-dimensional gradient exists, then there should be a rotation that produces maximal diagonal dominance of the matrix. We quantified this by plotting the row elements of the connectivity matrix against its distance from the diagonal (Figure 7.4(d)) and then fitting an exponential decay model to the data defined by Equation 7.3, where y is the connectivity probability between both ROIs, x is their relative distance to the diagonal and b is the exponential decay parameter. The optimal angle is such that $1/|b|$ is maximum.

$$y = e^{-bx} \quad (7.3)$$

Figure 7.5 shows the re-ordered connectivity matrices, plots of connectivity versus distance from diagonal, and cost function values for rotation angles varying between -90 and 90 degrees. The optimal angle was found to be 80 degrees, which corresponds to the simulated angle.

Another approach that we explored is spectral reordering. This method exploits the use of eigenvectors to optimally cluster or re-order nodes (Higham et al., 2008; McDonald et al., 2012). Spectral-reordering can also be cast as a cost-function optimisation. Given a symmetric matrix, A with dimensions n by n , the cost function, C is given by

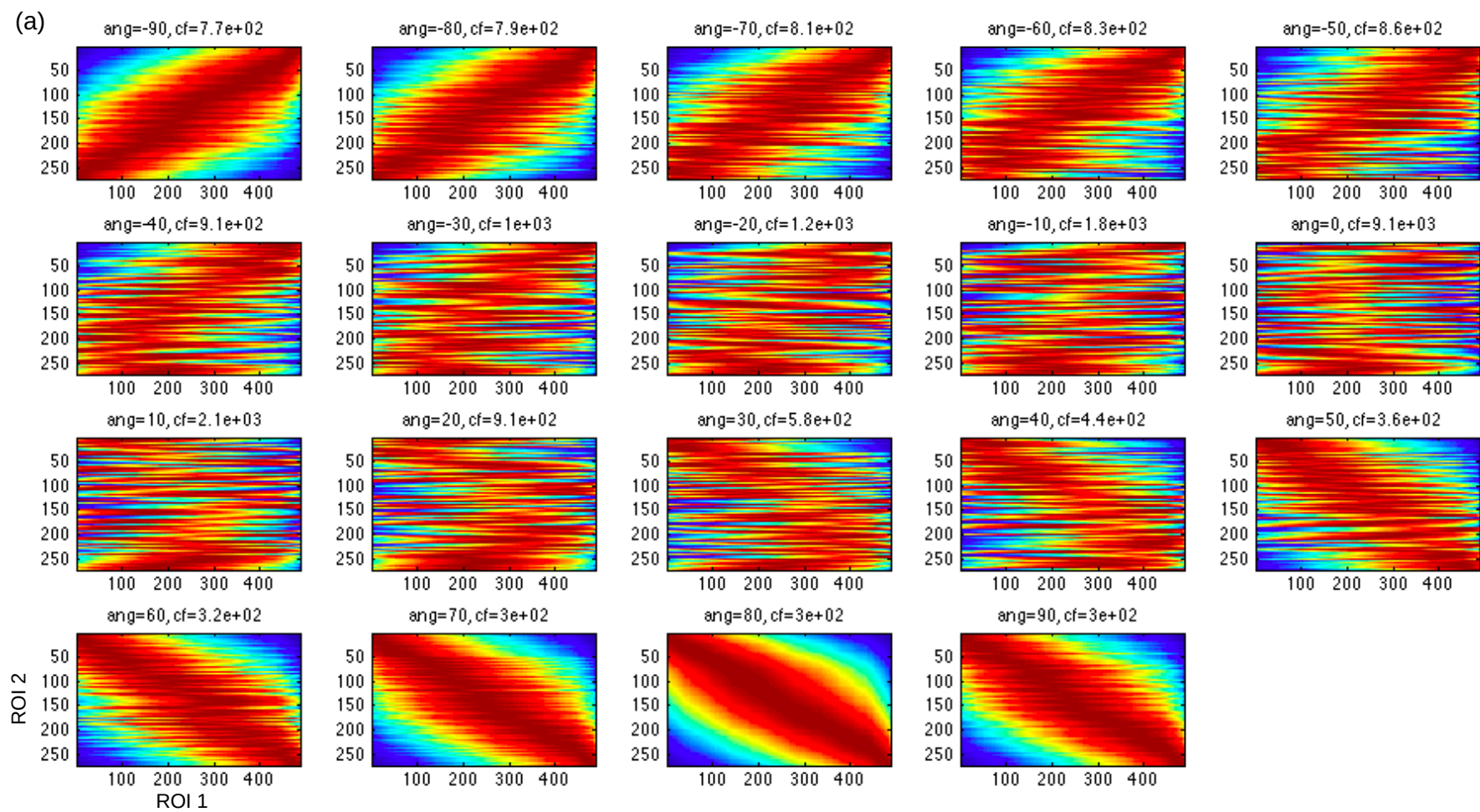
$$C = \sum_{i,j} (x_i - y_j)^2 A_{ij} \quad (7.4)$$

The aim is to minimise C and determine vectors x and y under the following constraints

$$\sum(x_i) = 0 \quad \sum(y_i) = 0$$

$$\sum(x_i^2) = 1 \quad \sum(y_i^2) = 1$$

This cost function is minimised when consecutive values of x and y correspond to high values in A , and therefore re-ordering the matrix according to values in x favours diagonal dominance. If A is a square matrix, $x=y$ can be computed by finding the principal eigenvector, however if A is rectangular then we must employ the singular value decomposition (SVD). Given an unordered rectangular gradient connectivity matrix (Figure 7.6), via spectral reordering we determined its optimal re-ordering. This information can then be combined with the spatial coordinates of ROI vertices to determine if the re-ordering corresponds to a simple rotation, in which case we can easily determine the angle of the rotation as in the cost-function method.



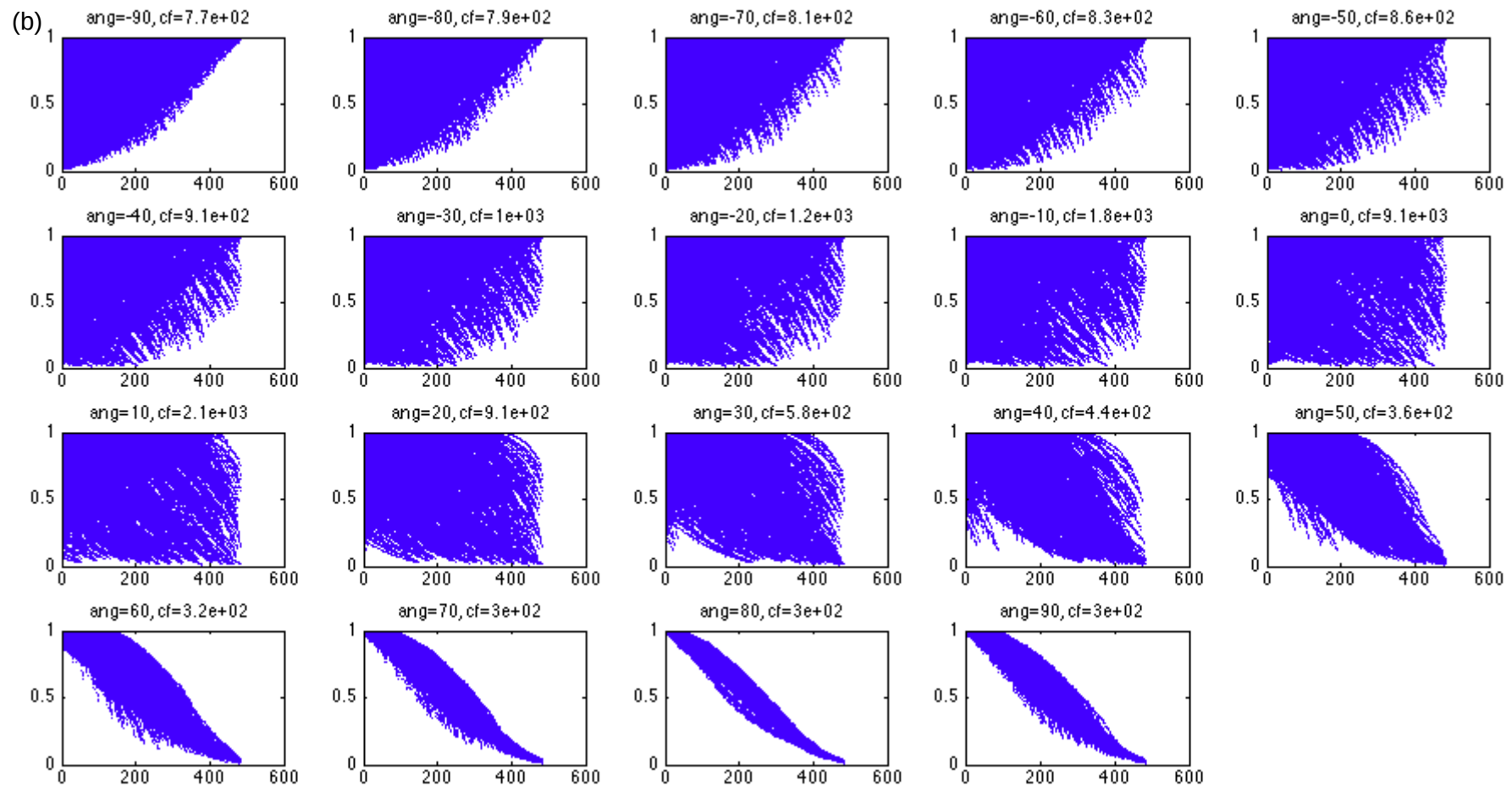


Figure 7.5. Detecting of gradient connectivity by computing a cost function. (a) Whilst one ROI was stationary the second was rotated by a range of angles and their gradient connectivity distribution constructed. (b) The distance between each voxel to the diagonal and an exponential decay model fitted to compute the cost function to determine the optimal angle between both ROIs. At this instance 80 degrees being the optimal angle.

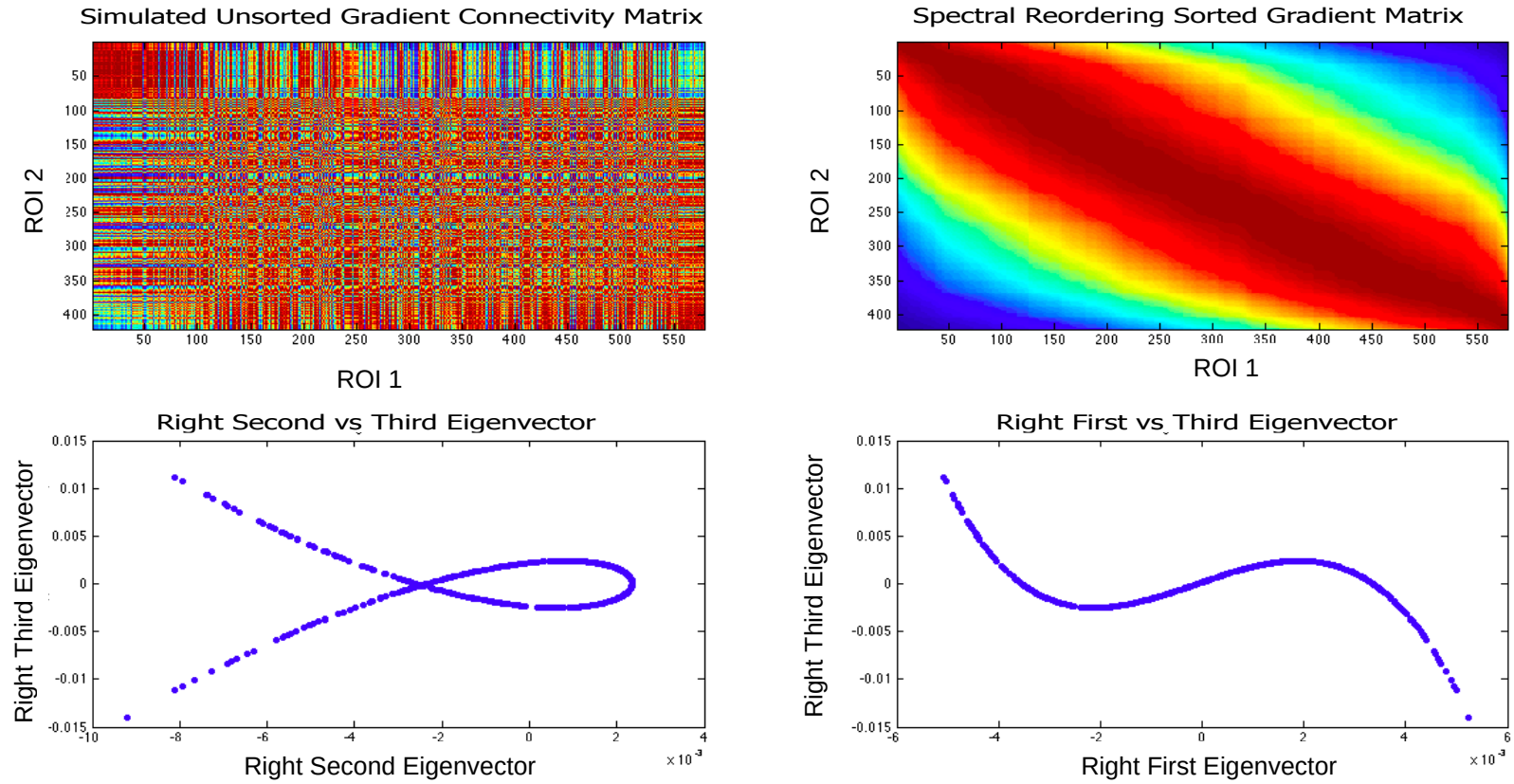


Figure 7.6. Detection of gradient connectivity matrix via spectral re-ordering. Given a simulated unsorted gradient connectivity matrix and via spectral re-ordering we re-ordered gradient matrix, optimally. The bottom images show the right top three eigenvectors computed from Singular Value Decomposition (SVD).

7.4 Discussion

Brain networks exclude and ignore the point-to-point mapping between regions, referred to as topography (Kaas, 1997; Thivierge and Marcus, 2007; Udin and Fawcett, 1988). Spatial arrangements between brain areas aim to reduce brain-wiring costs for efficiently performing complex behaviours (Jbabdi et al., 2013; Saygin et al., 2012). Examples of topography include retinotopic (Wandell et al., 2007), somatotopic (Stippich et al., 2002), and tonotopic (Saenz and Langers, 2014). These mappings are witnessed between sensory areas and the cerebral cortex (Humphries et al., 2010; Saenz and Langers, 2014), in addition to connectivity patterns between cortical and subcortical areas (Amaral et al., 2003; Rempel-Clower and Barbas, 1998; Suzuki and Amaral, 1994).

There is a range of topographic connection types, from cluster to gradient patterns (Schall et al., 1995; Suzuki and Amaral, 1994). We modelled and simulated spatial gradients of connectivity to study potential detection and classification of topographic connections. We mathematically defined gradient mappings and simulated such connectivity. We showed that these organisations could be recovered by minimising a cost function that calculates the optimal angle of rotation between two ROIs. Optimality was defined using the diagonal dominance of the connectivity matrix. In addition, we showed that a spectral re-ordering algorithm could also find diagonally dominant matrix organisation without information about the spatial layout of the regions. In general cost function and spectral clustering are methods that enable us to detect gradient patterns (Higham et al., 2008; McDonald et al., 2012), though

spectral re-ordering is much more robust to noise, and could be used to greater effect on real data.

Over the years, neuroimaging will certainly improve allowing for the probing and development of topography pattern detection and classification tools of the human brain. Brain networks should not be excluded, though topographic connections should be considered as an additional parameter to be taken into account. Future work includes producing a more detailed catalogue of different topographic connections that will enable us to characterise these patterns.

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Chapter 8

Human Amygdala Anatomical and Functional Parcellation

8.1 Introduction

The amygdala is an almond-shaped subcortical complex structure situated within the medial temporal lobe. It plays key roles in memory (Aggleton, 1992), learning and emotional processing (LeDoux, 2007). Evidence from histological studies suggests that the amygdala comprises of over 13 nuclei (Whalen and Phelps, 2009), though it can be clustered into two main subdivisions, the basolateral and centromedial. Attempts have been made to delineate the human amygdala via diffusion or functional imaging, but never both. We study the distinct cortical projections of the human amygdala, and apply a data-driven approach for functional and structural connectivity-based parcellation of the human amygdala. Following this, we compare the anatomical and functional connectivity of the human amygdala to explore how functional connectivity is shaped by anatomical connections.

8.2 Neuroanatomy of the amygdala

The amygdala derived its name from the Greek word meaning ‘almond’ due to its almond-like shape. The amygdala is part of the limbic system and located in medial temporal pole, as shown in Figure 8.1(a). Evidence from histological studies suggests that the amygdala comprises of over 13 nuclei (Whalen and Phelps, 2009) (Figure 8.1(b)), though this is still under much debate. Some

scientists claim that the amygdala belongs to other brain areas rather than a single functional or structural entity (LeDoux, 2007), although it is agreed that the amygdala can be clustered into two main subdivisions, the basolateral and centromedial complex. The basolateral complex consists of the lateral (L), basal (B) and accessory basal (AB) subnuclei, whereas the centromedial complex comprises of the central (C), cortical (Co) and medial (M) subnuclei (LeDoux, 2007)¹.

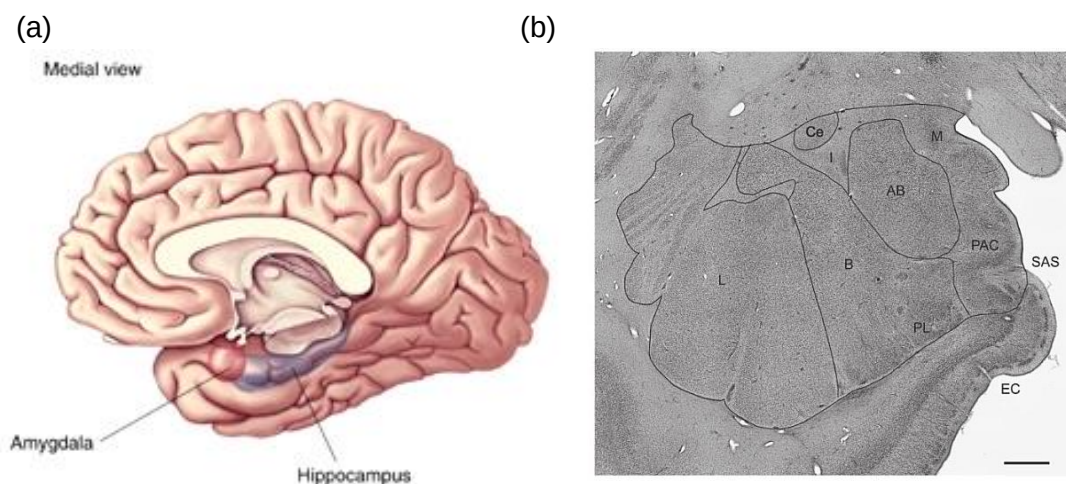


Figure 8.1. The amygdala (a) The amygdala is situated at the medial temporal pole with key functions in memory, learning and emotion. Taken from (Bear et al., 2007). (b) A Nissl-stained coronal view of right human amygdala subdivisions with scale bar of 2mm. Borderlines represent subnuclei regions. Main amygdala subnuclei are lateral (L), basal (B), central (C), accessory basal (AB) and medial (M). Taken from (Whalen and Phelps, 2009).

The amygdala subnuclei are functionally and structurally distinct (Saygin et al., 2011), but share intra-amygdaloid connections with one another (Sah et al., 2003). The lateral nucleus is the main input recipient and the central nucleus is the main output to other brain regions (LeDoux, 2007). Based on histological tracers, connectivity patterns, and cytoarchitectural studies the

¹ Excluding cortical subnuclei from study due to its ambiguity of being part of the amygdala

amygdala projects to and from a range of cortical and subcortical brain areas, evident from primate (nonhuman) studies (Table 8.1) (Sah et al., 2003).

Table 8.2. Anatomical binary connectivity matrix between the amygdala (seed) and other brain regions (target) based on primate (nonhuman) histological studies. Red indicated strong connections, and yellow signifies weak projections between seed and target.

Amygdala Subnuclei/ Target Region ¹	Cental (C)	Medial (M)	Lateral (L)	Basal (B)	Accessory Basal (AB)
Anterior Cingulate Cortex (ACC) ²	0	0	1	1	1
Frontal Orbital Cortex (FOC) ³	0	0	1	1	1
Frontal Medial Cortex (FMC) ³	0	1	0	1	1
Insular Cortex (IC) ³	0	1	1	1	1
Intracalcarine (Intra) ⁴	0	0	0	1	1
Occipital Pole (OP) ⁴	0	0	0	1	1
Postcentral (Post) ^{4,5}	0	0	0	1	0
Accumbens (Accum) ⁶	0	0	0	1	0
Caudate (Caud) ⁷	0	0	1	1	0
Temporal Fusiform (TF) ^{2,4}	0	0	0	1	1
Temporal Pole (TP) ^{2,4}	0	0	0	1	1
Thalamus (Thal) ⁴	1	0	1	1	1
Brain Stem (Stem) ⁸	0	1	0	1	1

References
¹ (Whalen and Phelps, 2009)
² (Amaral and Insausti, 1992)
³ (Leichnetz and Astruc, 1976a; Leichnetz and Astruc, 1976b; Leichnetz et al., 1976)
⁴ (Aggleton et al., 1980)
⁵ (Stefanacci and Amaral, 2000)
⁶ (Russchen et al., 1985)
⁷ (Fudge et al., 2002)
⁸ (Mehler, 1980)

8.3 Current studies of parcellation

Attempts have been made to anatomically parcellate the amygdala. Constructing probabilistic maps by registering subjects to a common anatomical reference space is prone to normalisation errors (Amunts et al., 2005). Clustering heterogeneous amygdala voxels via PDD is restricted only to the centromedial and basolateral complexes, hence excluding the amygdala's subnuclei (Solano-Castiella et al., 2010). Manually parcellating the amygdala based on a single subject study is also human error prone and very inaccurate (Etkin et al., 2004). Anatomical connectivity based probabilistic tractography has recently enabled us to segment the amygdala (Bach et al., 2011; Saygin et al., 2011), as well as with other subcortical structures, such as the thalamus (Behrens et al., 2003a).

Saygin et al. (2011) is the most coherent amygdala parcellation study to date.

The team developed a Tractography-based Segmentation (TractSeg) tool to parcellate the amygdala into four subnuclei, assuming the basal and accessory basal subnuclei are clustered into one anatomical region. The study was performed in native-space (Fischl et al., 2002; Fischl et al., 2004). Based upon prior knowledge of the connectivity patterns between the amygdala's subnuclei and other cortical areas, boolean expressions were devised to define how these subnuclei connect to such areas. For instance, the lateral subnuclei project to the Temporal Pole or Fusiform, but not Superior Parietal. The boolean expressions were applied to probabilistic tractography for classification of each voxel into one of the four amygdala subnuclei regions. Voxels that did not fit into any expressions were excluded from the study and only a limited number of target regions were employed.

Fewer studies have been conducted in functionally parcellating the amygdala via resting-state fMRI. Mishra et al. (2013) applied R-fMRI data to group correlated amygdala voxels into two subdivisions, via a self-organising map (Mishra et al., 2013).

8.4 Method

We functionally and anatomically parcellated the amygdala using the high quality Human Connectome Project (HCP) data (Smith et al., 2013; Sotiropoulos et al., 2013b; Van Essen et al., 2013)². The amygdaloid complex projects to cortical and subcortical brain areas, evident from histological tracer studies (Table 8.1). Based on these connectivity patterns, we applied

² Data were provided [in part] by the Human Connectome Project, WU-Minn Consortium (Principal Investigators: David Van Essen and Kamil Ugurbil; 1U54MH091657) funded by the 16 NIH Institutes and Centers that support the NIH Blueprint for Neuroscience Research; and by the McDonnell Center for Systems Neuroscience at Washington University.

probabilistic tractography and fMRI seed-based correlation analysis between target and seed regions to group voxels into spatially distinct areas.

Subjects: We analysed diffusion and R-fMRI images in 40 and 131 healthy unrelated subjects (aged 22-35), respectively, part of the first quarterly data release from the WashU-UMinn-Oxford HCP.

Masks: We outlined the amygdala masks in each subject using the model-based segmentation tool, FIRST (Patenaude et al., 2011) (Figure 8.2). Target masks were based on the Harvard-Oxford atlas in MNI 152 standard space. The Harvard-Oxford atlases consist of 48 cortical and 21 subcortical structural areas constructed from probabilistic atlases and obtained from T₁-weighted images (Desikan et al., 2006; Frazier et al., 2005; Goldstein et al., 2007; Makris et al., 2006)³.

³ We are very grateful to the following for providing the segmentations used to create these atlases: David Kennedy and Christian Haselgrove, Centre for Morphometric Analysis, Harvard; Bruce Fischl, the Martinos Center for Biomedical Imaging, MGH (NIH grants P41-RR14075, R01 RR16594-01A1, R01 NS052585-01); Janis Breeze and Jean Frazier from the Child and Adolescent Neuropsychiatric Research Program, Cambridge Health Alliance (NIH grants K08 MH01573, K01 MH01798); Larry Seidman and Jill Goldstein from the Department of Psychiatry of Harvard Medical School.

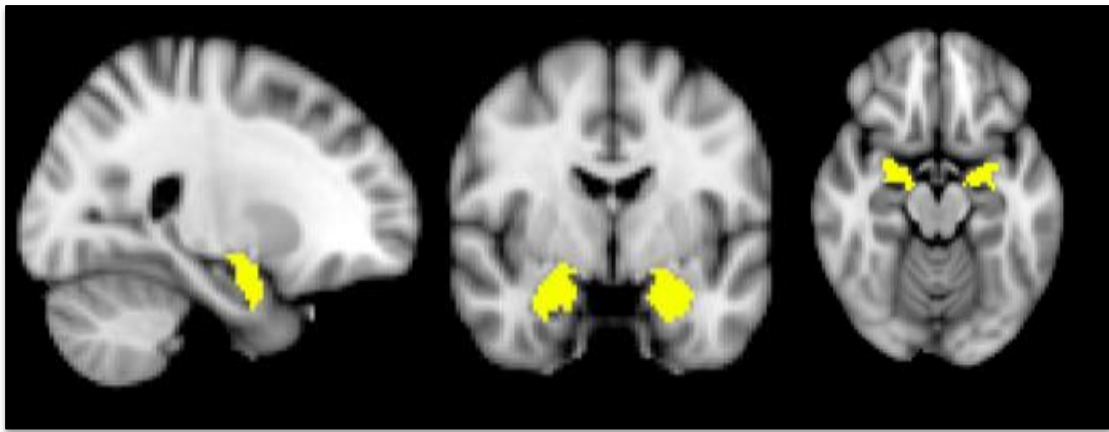


Figure 8.2. Amygdala seed masks. Left and right amygdala seed masks extracted using FSL FIRST based on the MNI 2mm anatomical standard space.

Diffusion Data Acquisition and Pre-processing: Diffusion-weighted images (DWI) were acquired using Spin-Echo (SE) EPI sequence (TE= 89.5 ms, TR= 5520 ms, echo spacing=0.78ms, 168x144 matrix, flip angle=78 deg, 111 slices, voxel size = 1.25 mm, isotropic, multiband factor=3, bandwidth=1488 Hz/Px, phase partial fourier=6/8) with 90 diffusion gradient directions ($b=1000, 2000, \text{ and } 3000 \text{ smm}^{-2}$). Six volumes without diffusion weighting were acquired ($b=0$) (Sotiropoulos et al., 2013b). Diffusion data were pre-processed using the pipelines described in Glasser et al (2013) (Glasser et al., 2013). This included intensity normalisation across runs. Correction techniques comprised of EPI distortion correction via TOPUP algorithm (Andersson et al., 2003), eddy current and motion correction using EDDY, and gradient non-linearity correction. Diffusion data was mean b_0 registered to native volume T_1 -weighted images using FMRIB's Linear Image Registration Tool, FLIRT and Boundary-Based-Registration, BBR. This was followed by MNI space transformation.

Probabilistic Tractography: For each subject, BEDPOSTX was applied to estimate brain voxel crossing fibres for a maximum of two fibre orientation per

voxel (Behrens et al., 2007; Jbabdi et al., 2012). FSL probabilistic tractography, probtrackx2 was employed in MNI 152 2mm standard space with 25000 samples per voxel (Behrens et al., 2007). A termination mask was also applied to stop tracts passing through the thalamus and brain stem as to halt indirect tracts to the amygdala (Bach et al., 2011). This termination mask was added to all target regions, excluding the brain stem and thalamus masks. Probtrackx2 estimates the probability of connectivity between each ipsilateral target (from Table 8.1) and seed (amygdala) voxel by determining the number of traces arriving at each target regions. We also calculated path distribution between seed and ipsilateral target regions, for the right and left amygdala individually. The connectivity and path distribution between each seed voxel and target region was then averaged across the 40 subjects.

Figure 8.3 illustrates an example of the average connectivity and path distribution between the amygdala and occipital pole, thresholded to 0.1. The basal subnuclei shows highest connectivity to the target, measured against volumetry human amygdala studies (Figures 8.3(a) and (c)). The basal subnuclei and occipital pole communicate via the Inferior Longitudinal Fasciculus (ILF), evident from our study (Figure 8.3(b)) and macaque studies (Figure 8.3(d)).

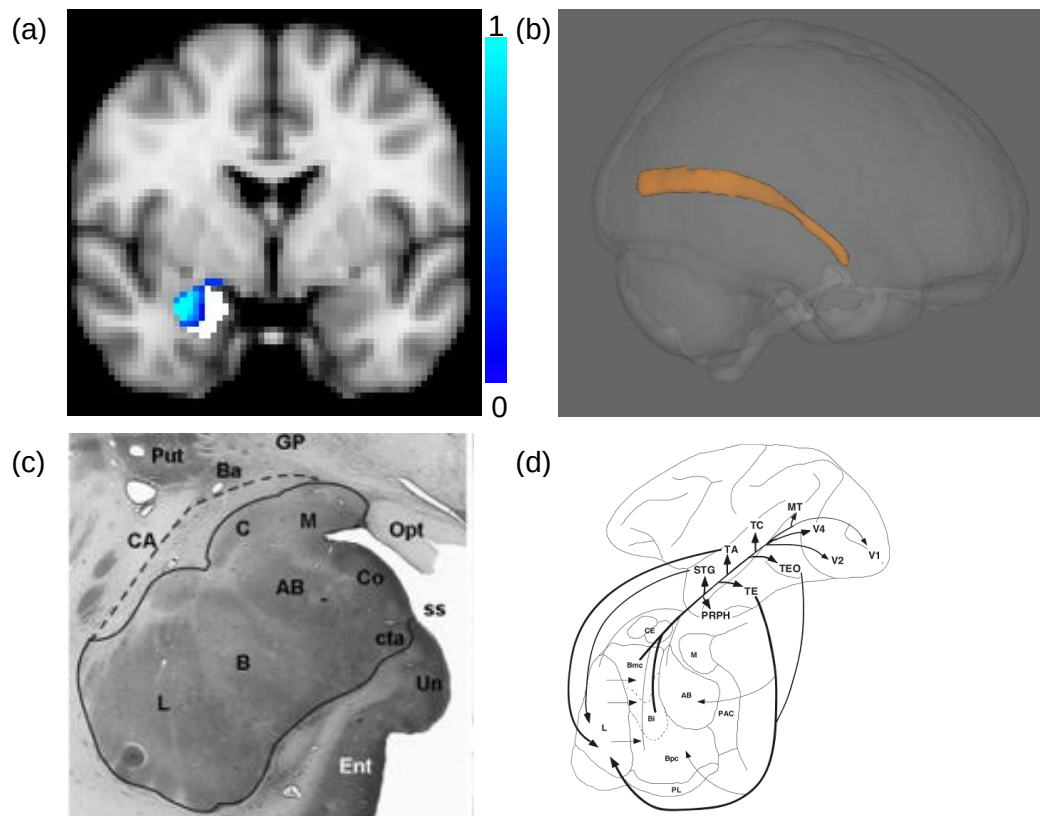


Figure 8.3. Example of the average connectivity and path distribution between the amygdala and occipital pole (a) Average connectivity distribution between amygdala and occipital pole, thresholded to 0.1. White region refers to amygdala seed mask. Highest connectivity located at the basal subnuclei analogous to human amygdala studies (c). (b). Path distribution based on probabilistic tractography between seed and target region via Inferior Longitudinal Fasciculus (ILF). (d). ILF pathway comparable to macaque studies. (c) Taken from (Brabec et al., 2010) (d) Taken from (Whalen and Phelps, 2009).

Resting-State fMRI Data Acquisition and Pre-processing: R-fMRI data was acquired using Gradient Echo (GE) EPI sequence (TE= 33.1 ms, TR= 720 ms, echo spacing=0.58ms, 104x90 matrix, flip angle=52 deg, 72 slices, voxel size = 2 mm, isotropic, multiband factor=8, bandwidth=2290 Hz/Px) (Smith et al., 2013). Data was obtained in two sessions. Each session entailed a run in the right-to-left (RL) and left-to-right phase encoding directions. Subject's eyes were open and fixated on a bright cross hair against

a dark background. Pre-processing of R-fMRI data included gradient field distortion correction and FLIRT-based motion correction (Jenkinson and Smith, 2001; Jenkinson et al., 2002). TOPUP-based field map was employed using SE field map, along with EPI to T₁-weighted registration via FLIRT BBR (Glasser et al., 2013). R-fMRI data was then resampled to atlas space and intensity normalised. A biased field removal was also applied via FSL to correct for RF inhomogeneities (Glasser et al., 2013).

Seed-Based Correlation Analysis: FSL `fsl_sbca` performs voxelwise seed based correlation analysis (Mars et al., 2011; O'Reilly et al., 2010). From each mask (target and seed), we extracted the R-fMRI voxelwise time series and computed its first Eigen time series to determine the overall coherent behavior of the mask (i.e. the largest variance across the mask's voxels) (Mars et al., 2011). For each subject, via `fsl_sbca` we computed the cross-correlation between each seed voxel and target first Eigen series (O'Reilly et al., 2010), and averaged the results for each target region across the 131 subjects.

Clustering: The cross-correlation between connectivity distribution of all seed and target voxels was computed for diffusion and R-fMRI matrices independently (Johansen-Berg et al., 2004; Johansen-Berg et al., 2005). Two approaches were applied to parcellate the amygdala from the connectivity distribution matrices.

The first methodology is purely data-driven, and entails feeding each matrix into an automated clustering algorithm. Clustering algorithms aim to group

similar voxels and differentiate unlike clusters based on a criterion, such as distance (Hartigan, 1975). For instance, K-means is an unsupervised learning clustering algorithm. It clusters voxels based on their distance from clusters centroids, and is repeated until a convergence is reached (Lee et al., 2012; Lee et al., 2013). We segmented the amygdala into $K=2$ distinct clusters with the aim of separating the amygdala into the centromedial and basolateral subdivisions.

The second novel approach uses information of the amygdaloid complex anatomy to guide parcellation. Based upon our prior knowledge of the amygdala's cytoarchitecture, five predefined regions of a few voxels were constructed corresponding to the different amygdala subnuclei. For each predefined subnuclei region, we separately computed the average connectivity distribution between each of them and the target regions (based on Table 8.1). This provides a single value that best characterises the subnuclei's probabilistic connectivity to each target. We then computed the correlation coefficient between the average connectivity for each amygdala's predefined subnuclei mask and all the amygdala seed voxels. K-means clustering grouped the left and right amygdala into five and two distinct areas for diffusion and R-fMRI data, respectively.

8.5 Results

Figure 8.4 shows coronal slices of the left and right amygdala using K-means ($K=2$) and guided parcellation ($K=5$), compared to volumetric human study of the right amygdala (Brabec et al., 2010). At $K=2$, one cluster was found at

dorsolateral, dorsomedial and ventrolateral regions of the amygdala. This resembles the few millimetres wide centromedial division of the amygdala (in green). The clustering shows more correspondence to the central subnuclei than the medial. On the other hand, the remaining region (second cluster) corresponds to the basolateral subdivision.

From the second clustering approach, we segmented the amygdala into five distinct groups (Figure 8.4 and 8.5). The first cluster was located superficially at the dorsomedial extent of the amygdala, symbolic of the medial subnuclei (in brown). At the superficial level and dorsolateral region is a cluster comparable to the central amygdala subnuclei (in green). Together these correspond to the centromedial subdivision of the amygdala. The third region identified was the most ventrolateral of the amygdaloid, classified as the lateral subnuclei (Whalen and Phelps, 2009). Ventrally, we identified two clusters, one inferior to the other. These were characteristics of the basal and accessory basal subnuclei, represented in blue and aqua blue, respectively. This is the first time to our knowledge that we are able separate the basal and accessory basal, though further cross validation is required.

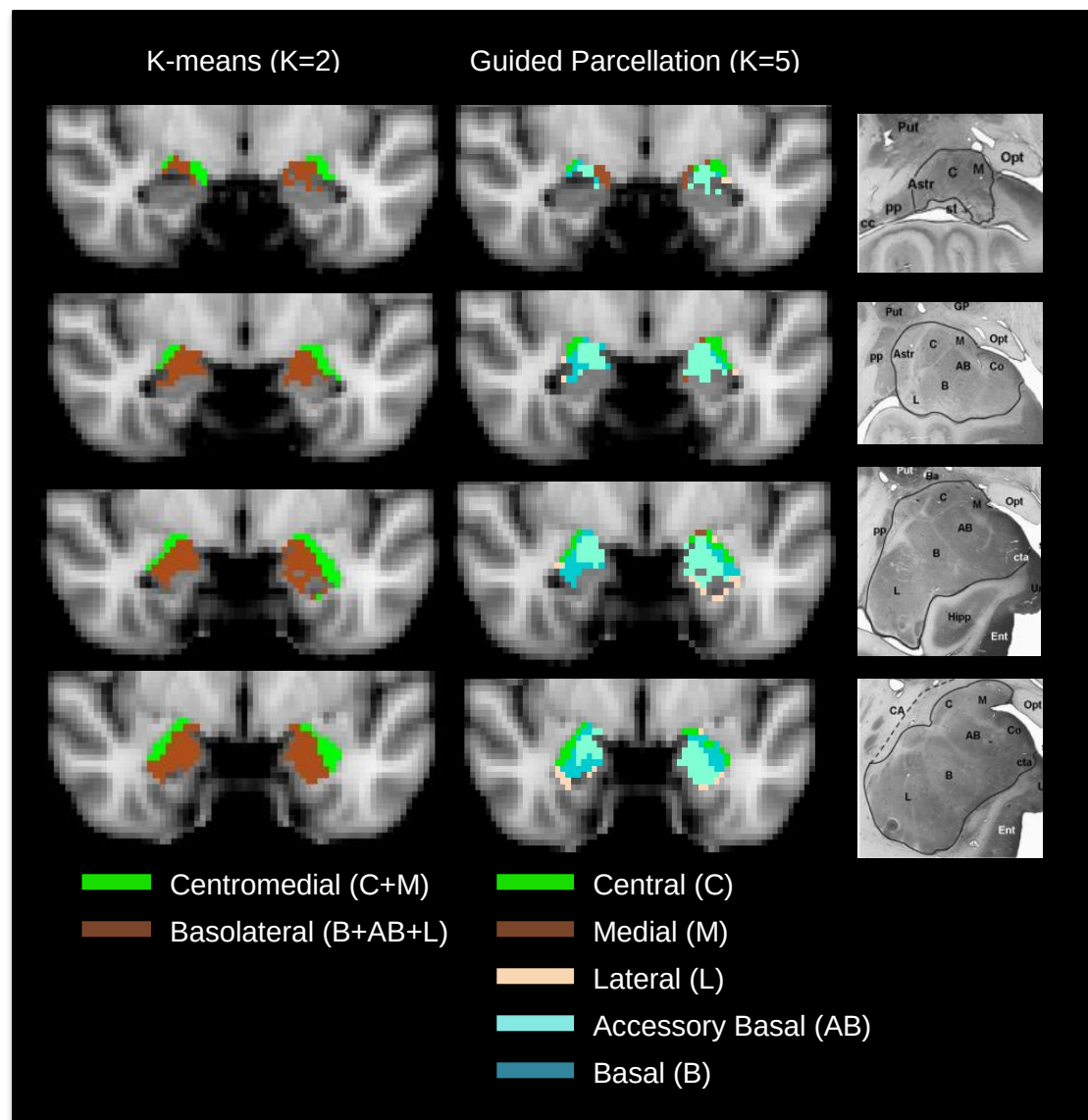


Figure 8.4. Tractography-based parcellation of the left and right amygdaloid complex. K=2 clustering of the amygdala into the centromedial (green) and basolateral (brown) subnuclei. K=5 anatomical guided parcellation segmented the amygdala into five subnuclei where brown is medial, green is central, cream is lateral, blue is basal and aqua is accessory basal. Both findings compared to human amygdala volumetry study of the right amygdala taken from (Brabec et al., 2010).

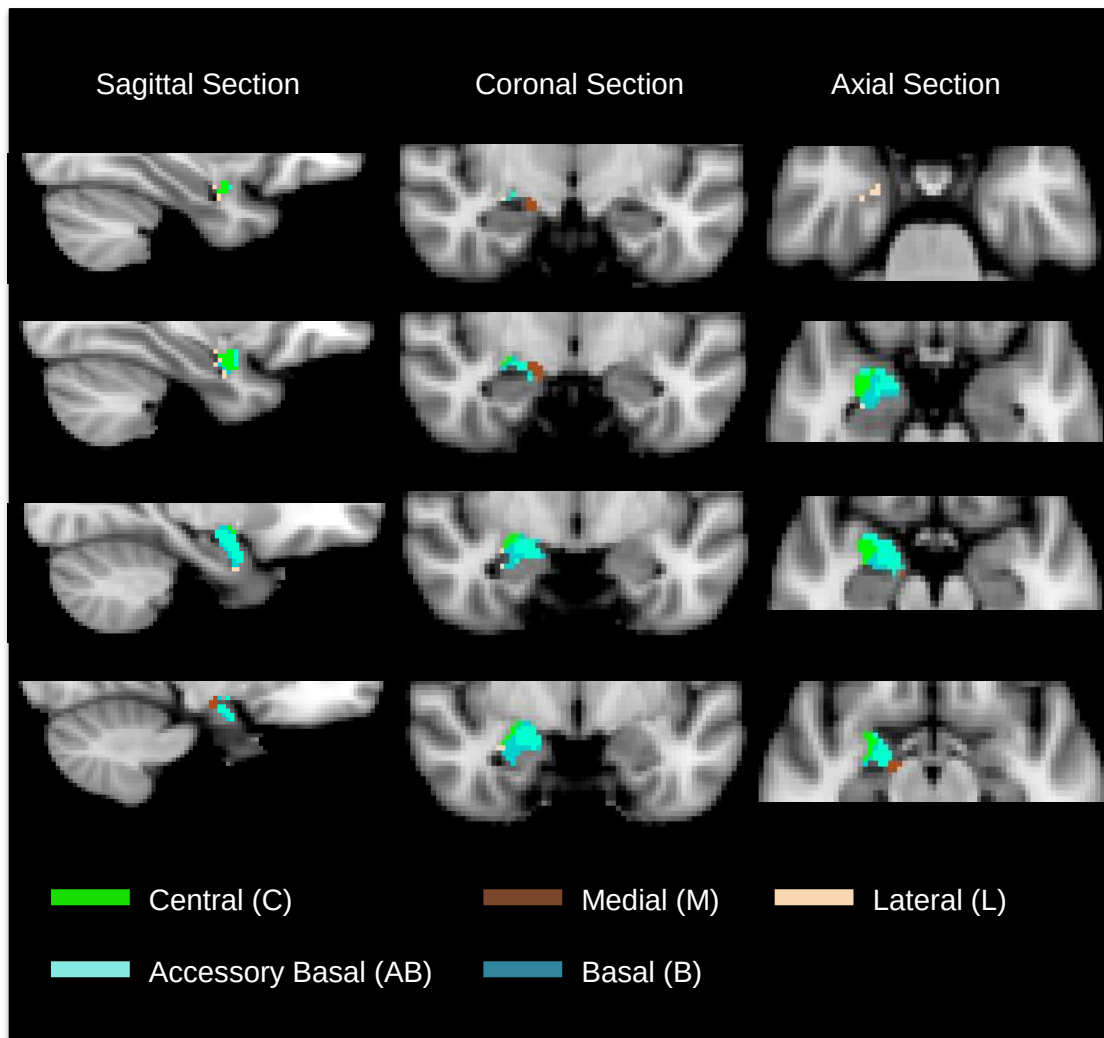


Figure 8.5. Parcellation of right amygdaloid complex for K=5 for different principal axes. Brown is medial, green is central, cream is lateral, blue is basal and aqua is accessory basal subnuclei. Sagittal sections show the medial subnuclei relative to other subnuclei. Coronal sections from posterior to anterior show the relative positioning of the accessory basal and basal subnuclei, as well as the central nuclei located more lateral than the medial subnuclei. Axial sections from inferior to superior illustrates the ventral positioning of the lateral, basal and accessory basal subnuclei in relation to the central and medial subnuclei of the amygdala.

Functional-based parcellation resulted in two regions identified (Figure 8.6). These divisions are consistent with the centromedial and basomedial regions outlined by cytoarchitecture. Using both methods for functional connectivity parcellation we achieved two clusters. One cluster (in brown) covered most of the amygdala, at the superficial and inferior level. This was similar to that of the basolateral division. In comparison, the second cluster (in green) was dorsolateral and dorsomedial superficial to the right and left amygdala, analogous to the smaller sized centromedial subdivision of the amygdala. Similar results were obtained using the anatomical guided parcellation compared to the human volumetric study (Brabec et al., 2010).

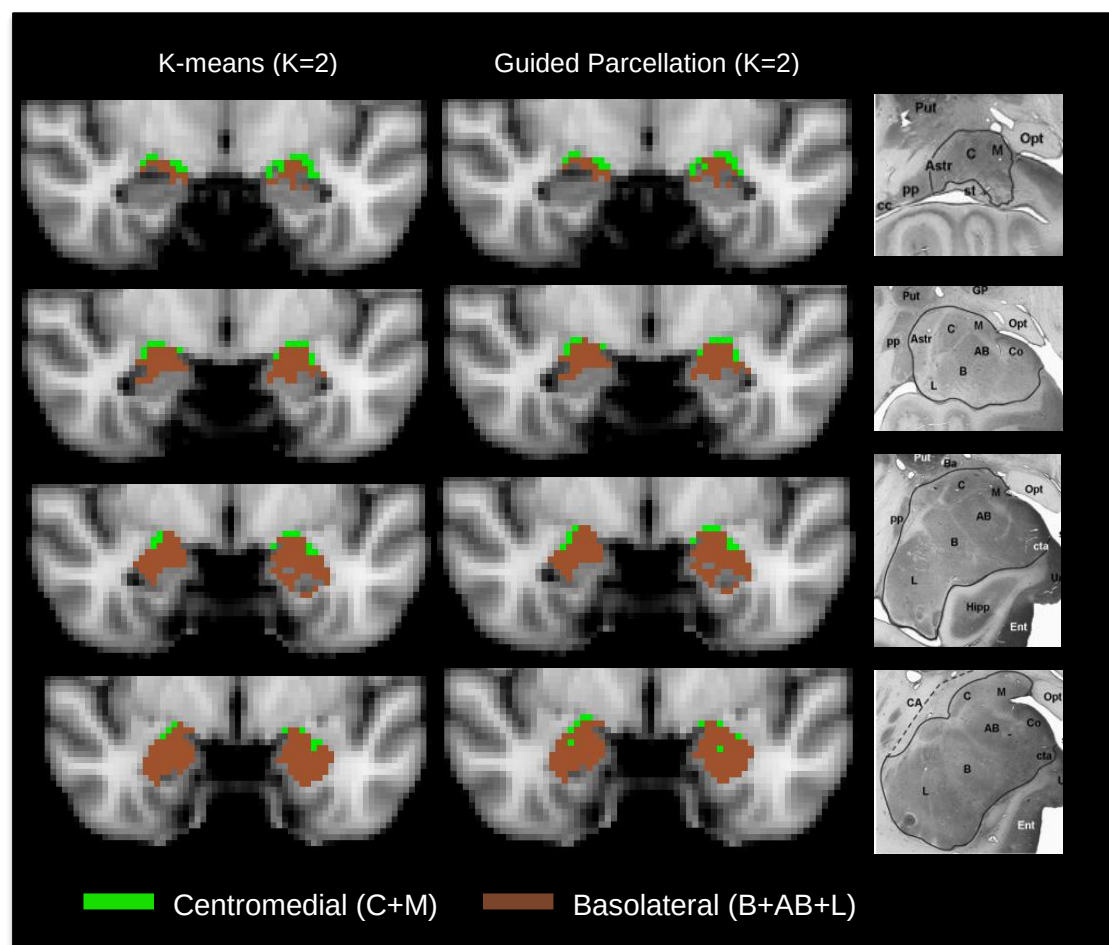


Figure 8.6. Functional-connectivity parcellation of the left and right amygdaloid complex. K=2 clustering and anatomical guided parcellation. Green is centromedial and brown is basolateral comparable to human amygdala volumetry study of the right amygdala taken from (Brabec et al., 2010)

8.6 Discussion

The amygdaloid complex is important in memory, learning and emotion (Aggleton, 1992; LeDoux, 2007). The amygdala consists of two main subdivisions, the centromedial and basolateral region consisting of five subnuclei, the central, lateral, basal, medial and accessory basal. We compared functional and structural connectivity of the brain at the cortical and subcortical level by parcellating the amygdala into its subnuclei via a data-driven approach, using the high quality HCP database (Van Essen et al., 2013). We extensively studied the unique projections between the amygdaloid subnuclei projections to other brain regions based on primate histological tracer studies (Table 8.1). Probabilistic tractography and voxelwise seed based correlation analysis were respectively applied to segment the amygdala functionally into the centromedial and basolateral, and anatomically into its five subnuclei.

Attempts of amygdala parcellation include the use of probabilistic maps (Amunts et al., 2005), principal diffusion direction clustering (Solano-Castiella et al., 2010), manual parcellation (Etkin et al., 2004), or probabilistic tractography (Bach et al., 2011). Functional connectivity parcellation methods include self-organising maps (SOM) to cluster correlated amygdala voxels (Mishra et al., 2013). To date, Saygin et al. (2011) is the most consistent approach for amygdala parcellation applied in native-space, however only segmenting the amygdala into its four nuclear groups (Saygin et al., 2011). Our study is the first case to our knowledge that anatomically differentiates the amygdaloid complex into its five spatially distinct constituents. Further

work is necessary to validate these findings. Similarities between the left and right amygdala were apparent from Figure 8.4 to 8.6, also consistent across subjects with minimal difference between both them. The centromedial and basolateral regions are analogous to both imaging modalities, highlighting evidence of the anatomical and functional connectivity relationship.

We executed the study in Montreal Neurological Institute (MNI) 152 standard space to delineate the target and seed mask, and perform parcellation. Registering subjects to a template anatomical space is susceptible to normalisation errors. It would be of interest to repeat this study in native-space to account for subject variation, tailored to each subject's anatomy (Fischl et al., 2008; Pujol et al., 2011). For instance, Table 8.1 showed the Frontal Medial Cortex projects to the basal nuclei, whereas the Brain Stem projects to the medial subnuclei. Comparing connectivity of average subjects to subject-specific studies, we could discover potential variations in subnuclei size, volume and individual connectivity changes, for further validation of our findings.

We employed K-means clustering and proposed a novel approach of anatomical amygdala guided parcellation. Our approach enables the use of a range of target regions, though adding more such as the Superior Parietal Cortex would enable more efficient automated parcellation of the amygdala. Based upon our prior knowledge of the amygdala's cytoarchitecture, five predefined masks of several voxels were constructed. A limitation is its high dependence to the amygdala subnuclei masks constructed by the user,

affecting clustering technique. Other methods independent of such masks and subject variation should also be considered for more robust amygdala segmentation, such as spectral reordering (Higham et al., 2008; McDonald et al., 2012).

Diffusion imaging cannot differentiate between afferent and efferent projections between seed and target regions. Nevertheless, in Chapter 6 we revealed distance dependence between regions of interest at the cortical level. A target closer to the amygdala has higher connectivity probability. Correcting for distance dependence and giving higher weighting to large scale connections enables us to remove biasing that should be eliminated for future parcellation techniques (Tomassini et al., 2007).

Further attempts need to be made to functionally parcellate the amygdala using R-fMRI as averaging time voxels in a target mask does not reveal detail about the amygdala subregions, rather only its general representation. Including task fMRI, we can determine probabilistic maps for heterogeneous clustering of the amygdaloid complex subnuclei, as well as identification of functional nodes that could further validate the results from the structural and resting-state data.

Meta-analysis has become a valuable asset over the years in combining independent statistical data from a range of studies (Egger et al., 1997). It can also be utilised alongside diffusion and functional data for comparing results from different amygdala studies and hence identifying patterns. Our study only

included primate histological tracer studies. Through meta-analysis we can explore human projections between the amygdala and other brain regions, as well as other mammals, including cats and rats to investigate potential evolutionary relationships.

We suggest that these methodologies are applicable to other brain areas that have subnuclei characteristics and unique connectivity projections to other cortical and/or subcortical structures, such as the thalamus, to understand their distinct subnuclei functions and structural similarities and differences.

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Chapter 9

Conclusion

Over the past decades, neuroimaging continues to rapidly progress and is increasingly applied in the clinic. A hot topic in neuroscience is the structural and functional connectivity of the human brain at a macroscopic level, and the extent to which anatomical connections constrain functional connections- the focus of this thesis. Non-invasive in-vivo imaging is a gateway to revealing insight to such questions in the human brain. We first introduced the theory of neuroanatomy and the concepts of MRI, including its uses in diffusion and functional MRI and investigated different types of brain organisations- topographic and clustered/nuclear. Both types of organisations exist in the brain and can be measured with in vivo techniques.

Functional connectivity is a reflection of the underlying structural connectivity, yet the relationship is unclear. We studied the functional and structural connectome, highlighting strong correlations beyond the mere effect of distance between brain regions. Initially, we discovered that the strongest correlation is between ROIs functional connectome and its own structural connectome, driven by a distance bias. Upon regressing out this distance effect via the Rational Quadratic model the overall structural-functional correlation remains with significant changes in correlation in certain regions of the human brain prior and after regression, such as the dorsal posterior

cingulate cortex. These correlations are suggested for efficient and robust human brain wiring.

Next, we considered the idea that connections can be topographically organised, a feature that, while evident in sensory connections, is often ignored in brain connectivity research. There is a range of topographic connection types, from cluster to gradient patterns. We modelled such topographic connections and proposed an algorithm for the detection of topographic organisation in the human connectome. Our work encourages the inclusion of topographic connections as an additional parameter for future probing and development of brain networks.

Finally, we investigated the anatomical and functional subdivision of the human amygdala, and parcellated the amygdaloid complex into its subnuclei via probabilistic tractography and voxelwise seed based correlation analysis, also applicable to the subcortical structures. Histological studies show that the amygdala comprises of the basolateral and centromedial, which in turn concludes of five subnuclei; lateral, basal, accessory basal, central, and medial. Based on the unique projections between the amygdaloid subnuclei to other brain regions from primate studies, our Human Connectome Project (HCP) data-driven approach is the first case to our knowledge that anatomically differentiates the amygdaloid complex into its five spatially distinct constituents, with similarities between the left and right amygdala. The centromedial and basolateral regions were analogous to both imaging

modalities, highlighting evidence of the anatomical and functional connectivity correlation at the subcortical level.

MRI advancements have provided the ability to research the structural and functional similarities and differences of the human brain. It is certain it will continue to play a major role in neuroscience with continued development in this field. The structural-functional connectome needs to be further studied including more research into analysis techniques of the connectome, with the aid of different neuroimaging techniques, such as MEG as no one modality is likely to provide the complete solution. Overall, diffusion imaging and functional MRI will most certainly be the future and this thesis has offered approaches into understanding the relationship between structure and function of the human connectome.