Cortical structure
Linking MRI and cytoarchitecture

Konrad Wagstyl

MRI provides a powerful tool to investigate brain structure in living humans. However a major challenge is interpreting the biological underpinnings of changes at this scale. This dissertation describes investigations into the problem of linking microscale post mortem cortical cytoarchitecture with millimeter-scale measures of cortical anatomy accessible through in vivo MRI.

Chapter 1 introduces the problem and previous work done to address it. The following two chapters apply classical atlases of cortical cytoarchitecture to understanding morphological changes both in health (Chapter 2) and in disease (Chapter 3). Chapter 2 demonstrates that sensory processing hierarchies exhibit increasing gradients of cortical thickness, related to changes in cortical cytoarchitecture. In Chapter 3, cytoarchitectonically described differences in gyral and sulcal laminar structure were used to create markers of laminar change from MRI changes in schizophrenia.

Classical measurements of histology have limitations; they are observer dependent, two-dimensional with limited coverage of the cortex. To address these issues, Chapters 4-6 document work carried on BigBrain, a 3D 20μm resolution histological dataset. I created a high-resolution 3D atlas of laminar cytoarchitecture, which was mapped to MRI-compatible cortical surface reconstructions. Chapter 4 records the development of an automated 1D profile-based approach to laminar analysis, revealing basic principles of cortical cytoarchitecture. In Chapter 5 this approach was extended to identify 6 cortical layers throughout the isocortex. These tools can be used to segment 1D cortical intensity profiles derived from any modality. In Chapter 6, the analysis of cortical gradients initially identified using MRI cortical thickness in Chapter 2 was replicated and extended using novel histological data. First histological cortical thicknesses were tested for the same patterns organization measured on in vivo MRI in Chapter 2. These analyses were extended to test which layers contributed most to overall thickness. High-resolution, complete maps of cortical cytoarchitecture mapped to MRI-template cortical surface reconstructions, are a powerful tool and dataset for the neuroimaging community. They offer new possibilities for linking cortical microstructure to in vivo neuroimaging.
"First anatomy and then physiology; 
but if first physiology, then not without anatomy."

Korbinian Brodmann quoting Bernhard Gudden.

"The mind is a complex and many-layered thing, Potter.  
Or at least most minds are . . . "

Severus Snape,  
Harry Potter and the Order of the Phoenix,  
J.K. Rowling
Declaration

Research is an entirely collaborative, incremental endeavor and none of the work presented in this dissertation would have been possible without the many collaborators I have been fortunate to work with.

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This dissertation is my own work, except analyses done in collaboration with co-authors which are outlined below. This dissertation contains fewer than 65,000 words including appendices, bibliography, footnotes, tables and equations and has fewer than 150 figures.

Contributions by chapter:

Chapter 2 - Lisa Ronan advised on human FreeSurfer reconstruction of human MRIs and code for statistical tests. Sarah Beul carried out the FreeSurfer reconstruction of the macaque cortex, from which I extracted and analysed the thickness values. Ian M Goodyer and Paul C Fletcher provided edits and suggestions on the manuscript writing.

Chapter 3 - Lisa Ronan advised on FreeSurfer cortical reconstructions, analyses and interpretation, particularly of results relating to intrinsic curvature. Patient MRI and demographic data for this project were collected by collaborators from Oxford, Tim Crow and Neil Roberts. Ian M Goodyer and Paul C Fletcher provided edits and suggestions on the manuscript writing.

Chapter 4 - Claude Lepage provided technical support for using MINC tools and CIVET (the MNI neuroimaging and surface packages) and constant advice on automated surface extraction methods. Sebastian Bludau checked and edited manual layer segmentations of histological sections. Karl Zilles and Katrin Amunts gave advice on cytoarchitectural validation. Alan C Evans and Paul C Fletcher gave advice on study design and manuscript writing.

Chapter 5 - Claude Lepage provided technical support on handling data. Stephanie Larocque adapted the basic 1D convolutional network from existing 2D examples. Subsequent development and optimisation were carried out by myself, Stephanie, Guillem Cucurul
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Chapter 6 - Registration between FreeSurfer average surface template and the BigBrain
was carried out by Lindsay Lewis, which I subsequently used for mapping the Glasser atlas to
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Publications


Wagstyl K, Lepage C, Bludau S, Zilles K, Fletcher PC, Amunts K, Evans AC Mapping cortical laminar structure in the 3D BigBrain *In Submission*
**Conference Abstracts**

Wagstyl K, Ronan L, Beul S, Fletcher PC Cortical Thickness Predicts Functional hierarchy *Human Brain Mapping 2014*

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**Invited Presentations**

March 2015: “Markers of Supragranular thinning in Schizophrenia” Developmental Neurosciences, ICH.

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November 2015: “Cortical thickness gradients” NSPN Scientific Advisory Board Meeting, London


June 2016: “BigBrain: Laminar Structure” OHBM Oral presentation, Geneva

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December 2016: “BigBrain: Laminar Structure” Ludmer Center Scientific Advisory Board Meeting, Montreal

March 2017: "BigBrain: Linking cortical cytoarchitecture to MRI” Feindel Brain Imaging Lecture Series, MNI

August 2017: "Bringing BigBrain to Cambridge" Cambridge Connectome Consortium, UK
Chapter 1

Introduction

1.1 Relationships between cortical structure and function

Investigating the relationships between cortical structure and function has long been a fruitful avenue for understanding the brain at many scales. At the microscale level, Ramon Y Cajal hypothesised that brain was made up of a network of individual neurons and described patterns of interneuronal connectivity based on observations of individual stained neurons (Ramón and Cajal, 1904) (Figure 1.1). However, there is valuable insight to be had at a far coarser scale, for example the role of the frontal cortex in personality and decision making was elucidated by the unfortunate construction accident of Phineas Gage (Harlow, 1868), which involved dynamite and a 3-foot tamping iron bar. The bar entered and exited Phineas’s skull removing a portion of his left frontal cortex (Van Horn et al., 2012), which reportedly caused distinct personality changes. Thus there are invaluable discoveries to be made investigating the microscale principles of neurons and connections, and at the macroscale, which can be linked to behaviour and cognitive brain function.

Indeed this is no less true today where there are constant ongoing advances in both micro- and macro-scales of neuroscience. In vivo imaging proffers the capability to scan thousands if not hundreds of thousands of living individuals (Miller et al., 2016; Van Essen et al., 2013), investigating functional localisation (Glasser et al., 2016) or pathologies with numbers that were unthinkable perhaps 20 years ago (Di Martino et al., 2014). In parallel, huge amounts of microscale data are being made publicly available (Amunts et al., 2013; Shen et al., 2012), which allow large-scale exploration of genetic, cellular and cytoarchitectonic data. Individually these datasets offer great potential for new understanding, but to date research is unable to fully bridge the gap between micro- and macro-scale data. Thus a real outstanding challenge in neuroscience is creating the tools and frameworks for translating between the spectrum of available data. Overall, the work in my thesis aims to confront the
Fig. 1.1 Drawings of cortical neurons by Santiago Ramon y Cajal show complex, patterns of neuronal morphology and connectivity revealed by Nissl-staining cell bodies in the visual (left) and motor (middle) cortices, and a Golgi-stained cortex (right). (y Cajal, 1899).
problem of linking post mortem microscale cortical cytoarchitecture and millimeter-scale measures of cortical anatomy accessible through in vivo MRI.

1.2 Cortical anatomy

The cerebral isocortex is a six-layered structure, on the outer surface of the brain (Zilles et al., 2015a). It is involved in many of our complex cognitive processes, including high-order visual processing, language and social interactions. Damage to the cortex can therefore lead to a wide variety of neurological and neuropsychiatric conditions.

From the exterior, the human cerebral cortex appears highly convoluted, in part to accommodate a surface area which has greatly expanded over the course of evolution (Zilles et al., 2013). Relative to mice the surface area in humans is 600-times greater, while the thickness has only increased 4-fold (Mota and Herculano-Houzel, 2015; Rakic, 2009).

The outward folds of the cortex are termed gyri, while the inwards folds are sulci. The morphology, or shape, of the cortex is relatively consistent within species. Primary folds are the most consistent and correspond most consistently to boundaries between structurally and functionally distinct areas (Fischl et al., 2008). Secondary folds, forming later in development are less consistent and tertiary folds even less so (Amunts et al., 2007b). Nevertheless the general consistency of the folds allows them to be used as anatomical landmarks. At the broadest level, the cortex is subdivided into four main lobes - frontal, parietal, temporal, occipital, alongside limbic and insular lobes (Zilles et al., 2015a)(Figure 1.2). Within lobes there are various parcellation schemes to further classify individual gyri and sulci (Desikan et al., 2006; Tzourio-Mazoyer et al., 2002).

1.2.1 Cortical layers

The isocortex has a broadly consistent structure with six main layers, which are visible as tangential bands on cell-stained histological preparations and are identifiable due to characteristic differences in neuronal morphology, density and arrangement (von Economo and Koskinas, 1925)(Figure 1.3). Layer I, the molecular layer, is relatively cell-sparse with few neurons and glia. Layer II, the external granular layer, is a much more dense band of small granular cells. Layer III, the external pyramidal layer, is characterised by large pyramidal neurons that become more densely packed towards its lower extent. Layer IV, the internal granular layer (usually referred to simply as the “granular layer”), generally contains only granular neurons, bounded at its lower extent by pyramidal neurons of layer V. Layer V, the internal pyramidal layer, contains large but relatively sparse pyramidal neurons
Introduction

Fig. 1.2 Parcellating the cortex into lobes and Brodmann areas (From (Zilles et al., 2015a), originally (Brodmann, 1909)). The frontal lobe is made up of the red/orange/pink areas, anterior to the central sulcus. The parietal lobe is posterior to the is in blue, occipital lobe is marked in yellow, the temporal lobe in green and the limbic cortex is in purple. The insula is not visible here, being buried by lateral parts of the frontal, parietal and temporal cortices but is roughly located medial to Brodmann areas 41, 42, 43 and 44.

while layer VI, the multiform layer, has a lower density of pyramidal neurons (Brodmann, 1909). This structure varies from region to region but these features can generally be used to identify layers on high-resolution histological preparations across the cortex. Alongside these cellular differences, layers have many associated properties that covary, such as the connectivity (Lund et al., 1979), myelination (Nieuwenhuys et al., 2015) and neurotransmitter distributions (Zilles et al., 2002) (Figure 1.3).

1.2.2 Micro-columns and microcircuits

The cortex is thought to be organised radially into repeating columns. Evidence for the existence of a columnar organisation comes from developmental (Rakic, 1988), structural (Helmstaedter et al., 2007) and electrophysiological studies (Mountcastle et al., 1957). Developmentally, neuronal precursors destined for the cortex are produced in the ventricular, subventricular and outer subventricular zones of the fetal brain (Dehay et al., 2015; Rakic, 1988). These migrate radially to the cortical mantle populating the layers from inside to out. The first neuronal precursors arrive at what will become layer VI and the last layer to form is layer I (Rakic, 1974). Radially, neurons in a single ontogenetic column have been shown to originate from the same precursor cell (Kornack and Rakic, 1995).

Structurally, nano-scale reconstructions of columns in cortical samples show relatively discrete vertical organisation - for example in the rodent barrel cortex (Helmstaedter et al., 2007). However these are at a much greater scale than single ontogenetic columns, with many ontogenetic columns forming a single microcolumn or hypercolumn. Similar radially
Fig. 1.3 Neuronal morphology, cell body distribution and myeloarchitecture vary across the cortical layers (Vogt and Vogt, 1903).
organised terminations of axonal connections have been measured in high-order association cortices of primates (Bugbee and Goldman-Rakic, 1983).

Electrophysiologically, the cortex has been shown to have tangentially discrete receptive fields. For example, neurons in the same radial column of the cat somatosensory cortex, respond to a single peripheral sensory field (Mountcastle et al., 1957). In the primary visual cortex of the cat, radially the receptive fields are consistent, whereas there is a gradual progression tangentially across the cortex (Hubel and Wiesel, 1959, 1962). While there are several scales and experimental definitions of cortical columns, there remains strong evidence of a columnar organisation within the cortex.

The evidence for the micro-column and consistencies in connectivities and properties of individual layers has given rise to the idea of a canonical microcircuit, whose basic computational principles are repeated throughout the cortex (Bastos et al., 2012)(Figure 1.3). This hypothesis suggests that each layer plays a characteristic role in cortical computations. For example, layer IV is the main input layer, while layer II-III pyramidal neurons send efferent forward connections and layer V pyramidal neurons primarily send feedback connections to lower order cortical areas, subcortex and brainstem.

There is value in simplification to create a generalisable cortical microcircuit, however investigating the differences in columnar structure between cortical areas that correspond to functional specialisation is as important to our understanding of cortical function (Rakic, 2008).

1.2.3 Cortical areas

The laminar structure and functional activations of the cortex vary greatly from area to area. In the extreme cases, in the precentral gyrus, the primary motor cortex has no clearly visible layer IV and is thus considered “agranular”, while in the calcarine sulcus, the primary visual cortex has at least three if not more clearly visible sublayers within layer IV (von Economo and Koskinas, 1925). These are extreme examples, but hundreds of more subtle variations in cortical structure can be used to subdivide, or parcellate, the cortex (Brodmann, 1909; Schleicher et al., 2005). Similarly, the cortex can be subdivided based on functional properties, through direct or surrogate measures of neuronal activity in response to particular tasks or stimuli (Glasser et al., 2016). A simple example is the primary visual cortex which shows a non-specific Blood-Oxygen-Level Dependent (BOLD) fMRI response to visual stimuli, while the “face” area on the fusiform gyrus of the temporal lobe selectively responds to the presentation of faces (Grill-Spector and Malach, 2004; Kanwisher et al., 1997). While it is difficult to measure laminar structure and active function in the same individual, there
Fig. 1.4 The canonical microcircuit is a simplified model of cortical function, whose basic principles are thought to be present with minor variations throughout the cortex (Bastos et al., 2012). Within this circuit, individual layers have specific roles in computing and transmitting feedforward and feedback signals. Cytoarchitectonic differences are likely to underly interareal, function-specific differences in this circuit (Rakic, 2008).
is a strong body of evidence that many if not all the functional borders in the cortex have corresponding structural boundaries (Glasser et al., 2016; Gomez et al., 2017).

This gives rise to a fundamental question in neuroscience. Given that there is evidence for a basic 6-layered canonical microcircuit, but that the 6-layered structure varies from area to area in a manner that correlates with functional changes, what exactly is changing and why? Which of the subtle differences enable what is fundamentally the same constituent circuitry to express such a range of functions, from responding to basic stimuli to carrying out complex mathematics, composing a symphony or writing a novel?

1.3 Measures of cortical structure

1.3.1 Insights from histology

Linked cytoarchitectural properties

Prior to the invention of non-invasive techniques to measure cortical structure, insights into links between cortical structure and function originated from ex vivo histological techniques. For example, Brodmann and von Economo & Koskinas created atlases of cortical structure in the early 20th century that are still valuable one hundred years later (Brodmann, 1909; von Economo and Koskinas, 1925)(Figure 1.2). Both atlases contain the observation that the total thickness of the cortex is related to other properties cytoarchitectural and functional specialisation. In particular they observe that primary sensory regions were generally thinner than their surrounding cortex, while the precentral motor area is the thickest cortex in the brain. Alongside total cortical thickness measurements, von Economo & Koskinas reported densities and thicknesses of each cortical layer in a large number of regions, and devised a summary descriptor of five basic laminar structural types for the neocortex. Primary sensory cortices were given structural type five, due to clear laminar differentiation, secondary sensory areas, type 4, association cortices types 3 and 2 and agranular cortices type 1 (Figure 1.5). These structural types were subsequently replicated and extended in macaque cortex classifying a total of 8 structural types (Barbas, 1986; Sanides, 1972).

The same structural types were later shown to predict the pattern of connections measured with axonal tracers, to other regions. In both macaques and cats (Barbas, 1986; Beul et al., 2017, 2014), projections from high-ranking structural types (eg primary sensory areas) tend to originate in supragranular layers (layers II/III), while projections from lower structural types to higher types tended to originate from infragranular layers. Moreover these patterns of connections have been interpreted as indicating the positioning of sensory regions in cortical hierarchies (Felleman and Van Essen, 1991; Markov et al., 2014). Thus the connections with
Fig. 1.5 Cortical laminar structural types. The five major structural types identified by von Economo & Koskinas (von Economo and Koskinas, 1925). Primary sensory type five is found in the central sulcus (somatosensory), temporal Heschl’s gyrus (auditory) and calcarine sulcus (visual), while association cortex is predominantly types two and three. There is also a visible gradient of histological thickness through the manual drawings of the five structural types.
supragranular origin are termed feedforward, and tend to originate from lower sensory areas (perhaps confusingly with a higher structural type), while feedback, infragranular-originating connections are from higher-order areas (but with a lower structural type).

Finally, the laminar pattern and hypothesised position in a functional hierarchy can be tentatively linked to the morphology of individual pyramidal neurons in cortical areas. Layer III pyramidal neurons, the origin of feedforward efferent axonal connections have relatively small cell bodies in the primary visual cortex, with limited dendritic arborisation but become larger and more arborised across the visual pathways of the macaque (Elston and Rosa, 1997; Elston et al., 1999).

As such analyses of ex vivo histology have been extremely useful in linking microstructural properties to one another, and have even been extended to suggesting functional implications of these properties.

Cytoarchitectonic parcellation

Another key aspect of neuroanatomy is the segmentation of the cortex into areas. Histological parcellations divide the cortex into regions that are structurally homogenous and distinct from neighbouring areas based on a range of properties. Many of the more clear boundaries can be distinguished by eye on histological preparations, such as the boundary between the primary visual cortex, (V1/Brodmann Area (BA) 17/von Economo are OC), and the secondary visual cortex (V2/BA18/OB)(Figure 1.6). Here the clear sublayers of V1, are not found in V2, making segmentation of the areas a relatively straightforward task. With experience, more subtle subdivisions can be identified and manual expert delineations have been used to create many parcellation schemes for the cortex (Brodmann, 1909; von Economo and Koskinas, 1925).

More recently, there has been considerable effort to make these methods observer-independent, using statistical comparisons of image intensities in the cortex (Schleicher et al., 1999, 2005). Histological cortical profiles are extracted from the layer I/II boundary to the grey/white boundary, summarising density changes throughout the cortical column. Differences in cell densities, indicated by intensity changes in the profiles can be used to detect subtle changes at the boundaries between neighbouring cortical areas. Other cytoarchitectonic approaches incorporate differences in tracer-based axonal connectivity to distal regions as additional features for differentiating and characterising areas (Petrides and Pandya, 2002).

Mapping of cortical areas plays a crucial role in translating between neuroscientific modalities and scales. Histological boundaries have a close relationship with cortical morphology (Fischl et al., 2008; von Economo and Koskinas, 1925), such that specific boundaries
1.3 Measures of cortical structure

Fig. 1.6 Boundary between BA17 (V1) and BA18 (V2) (Amunts et al., 2000). There is a clear change in laminar pattern between these two regions, with the many sublayers of layer IV in V1 merging to form a single layer IV in V2.

and regions tend to occur in the same gyral/sulcal locations. While neuroanatomists argue that this relationship is inexact - borders can shift relative to the sulcal fundus (Amunts et al., 2007b) - the broad principle is crucial for linking to MRI-scale approaches, where morphology can be used to link macroscale changes to specific cytoarchitectonic regions and all their associated properties. Indeed, this assumption that function maps reasonably faithfully to structure is central to functional neuroimaging studies, notably when spatially overlapping activation across individuals is taken as a signature of functional overlap.

1.3.2 Insights from MRI

Histological approaches can provide an immense level of detail about cortical neuroanatomy, but are limited by being necessarily *ex vivo*. By contrast, MRI has a lower resolution - ranging from 1mm isotropic voxels down to around 200 microns at the current cutting edge of high-field MRI. However the major advantage is that these studies can be done *in vivo* and with large numbers of subjects.

Despite the lower resolution of MRI, one measure of cortical cytoarchitecture that is still directly measurable *in vivo* is cortical thickness. Early studies of cortical structure relied upon manual measurements of cortical thickness, but were able to replicate certain histological findings such as the distinctly thick precentral motor cortex, relative to the
particularly thin primary somatosensory cortex (Meyer et al., 1996). The advent of automated and freely available tools to segment of the grey and white matters surfaces has exponentially increased our capacity to measure cortical structure (Fischl and Dale, 2000; Lerch and Evans, 2005). Various software packages including FreeSurfer, ANTS and CIVET have been created to carry out automated thickness analyses. Of these FreeSurfer has been extensively compared to post mortem histology. Generally the agreement with automated and manual post-mortem measurement is good (Fischl and Dale, 2000; Song et al., 2015), however there are some known systematic biases such as underestimation of cortical thickness in highly myelinated areas (Glasser and Van Essen, 2011). Despite these minor reservations, automated neuroanatomical approaches are invaluable as they can indicate in vivo microstructural differences. For example, subjects trained on motor tasks such as learning to juggle, show longitudinal structural MRI changes in the motor cortex (Draganski et al., 2004). Moreover, in vivo structure can be linked to functional and even cognitive measures. A thin primary visual cortex with a large surface area, has narrower fMRI-based receptive fields that correspond to increased ability to detect horizontal separation in visual stimuli (Song et al., 2015). Similarly, one study has linked the development of functional specialisation between visual face and object recognition areas to changes in MRI-based tissue properties and, indirectly to histological differences between these regions (Gomez et al., 2017).

1.4 Thesis objectives

With the ever increasing amounts of data, there is a large number of relationships between cortical microstructure and in vivo structural measures of MRI that have yet to be discovered. Identifying and characterising these relationships will shed light on the neurobiological underpinnings of brain structure in healthy and clinical populations. The goal of my thesis has been to link understanding of microscale cortical cytoarchitecture to features measurable in MRI. This has involved the application of classical histological atlases to in vivo studies of cortical structure using MRI, and the development of tools to move beyond 2D histological region-based measurement to 3D, automated, whole brain, cortical surface-based analyses of cytoarchitectural data.

The first two chapters demonstrate the application of classical atlases of cortical cytoarchitecture to understanding morphological changes both in health (Chapter 2) and in disease (Chapter 3) in terms of underlying cytoarchitecture and neuropathology. This methodology of mapping parcellations from classical histological atlases to in vivo MRI was also incorporated into other collaborations investigating structural development in adolescent cohorts, and the potential microstructural causes underlying them (Whitaker et al., 2016).
1.4 Thesis objectives

However, classical measurements of histology have a number of limitations. First, most commonly used atlases are now nearly 100 years old. The original specimens are no longer in a condition to be reanalysed, thus the measurements cannot be repeated and verified. Second, Von Economo and Koskinas carried out quantitative histological measurements on around 50 regions, therefore analysis of these atlases are limited to this small number of samples. This becomes particularly unbalanced relative to the vast quantities of data available at MRI, where it is possible to acquire over 160,000 thickness measurements from a single hemisphere of one individual. Third, histological measurements are usually manual, with observer-dependent variations estimated at +/-0.5 mm for placement of the white matter boundary. Finally, as these are 2D sections of a 3D convoluted surface, thickness measurements in particular are associated with errors introduced by the deviation from perpendicularity of slice angles. In order to address these issues, Chapters 4-6 document work done at the Montreal Neurological Institute on the BigBrain, a 3D 20µm resolution histological dataset. The aim of this work was to create a high-resolution 3D atlas of laminar cytoarchitecture, which could be mapped to a cortical surface reconstruction suitable for translation to 3D MRI analyses. Chapter 4 records the development of an automated 1D profile-based approach to laminar analysis, revealing basic principles of cortical cytoarchitecture previously unmapped across the entire cortex, quantitatively verifying one of the hypotheses driving Chapter 3.

In Chapter 5, I worked on more complex, flexible machine learning tools to identify 6 cortical layers throughout the isocortex. To do this I collaborated with members of researchers from the Montreal Institute of Learning Algorithms (MILA) to adapt existing convolutional neural networks to the 1D profile problem and successfully trained them on a limited number of expert histological annotations. This approach created mesh reconstructions of the 6 isocortical layers in the BigBrain.

In Chapter 6, I replicated and extended the analysis of cortical gradients first carried out using MRI cortical thickness in Chapter 2, first verifying that MRI gradients of thickness did mark changes in the histological thickness of the cortex. I then investigated which cortical layers drove these gradients. The high-resolution, complete maps of cortical cytoarchitecture developed in this thesis, which have been mapped to MRI-template cortical surface reconstructions are a powerful new freely available tool and dataset for the neuroimaging community. They offer new possibilities for linking cortical microstructure to in vivo neuroimaging.
Chapter 2

Cortical thickness gradients in structural hierarchies

2.1 Abstract

MRI, enabling in vivo analysis of cortical morphology, offers a powerful tool in the assessment of brain development and pathology. One of the most ubiquitous measures used - the thickness of the cortex - shows abnormalities in a number of diseases and conditions, but the functional and biological correlates of such alterations are unclear.

If the functional connotations of structural MRI measures are to be understood, we must strive to clarify the relationship between measures such as cortical thickness and their cytoarchitectural determinants. I therefore sought to determine whether patterns of cortical thickness mirror a key motif of the cortex, specifically its structural hierarchical organization. I delineated three sensory hierarchies (visual, somatosensory, auditory) in two species - macaque and human - and explored whether cortical thickness was correlated with specific cytoarchitectural characteristics. Importantly, the effect of cortical folding which impacts upon thickness was controlled for as it may obscure regional differences.

The results suggest that an easily measurable macroscopic brain parameter, namely cortical thickness, is systematically related to cytoarchitecture and to the structural hierarchical organization of the cortex. Measurement of cortical thickness gradients may become an important way to develop our understanding of brain structure-function relationships. The identification of alterations in such gradients may complement the observation of regionally localised cortical thickness changes in our understanding of normal development and neuropsychiatric illnesses.
2.2 Introduction

Although elegant work has established an indisputable general relationship between cortical morphology, cytoarchitecture and function (Broca, 1861; Fischl et al., 2008) the precise nature of this relationship is unclear. Moreover there is a considerable degree of inter-individual variability in the large-scale structural features of the brain, which adds further ambiguity to regional analysis of both structure and function (Amunts et al., 2007a; Rajkowska and Goldman-Rakic, 1995). The goal of the current study was to determine whether one widely used morphological measure - cortical thickness - varies across the cortex in a manner predicted by other cytoarchitectural and functional characteristics. Specifically, existing knowledge about the cellular, laminar and hodological patterns within the cortex were used to determine whether cortical thickness measures followed a corresponding pattern. Such an observation would lend important weight to the proposition that macroscopic structural measures obtained using MRI, act as a useful marker of underlying neurophysiology. If true then individual differences in cortical thickness measures would offer a possible interpretational framework for the neural bases of psychiatric disorders.

Central to this investigation is the principle that the brain processes information through pathways known as functional hierarchies. Computational hierarchical models describe a system of functionally specialized regions with feedforward and feedback connections (Rao and Ballard, 1999). Feedforward connections communicate incoming sensory information, while feedback connections relay experience-derived predictions (Bastos et al., 2012), which alter the response to the incoming signal (Bullier et al., 1996). Each cortical region processes particular features of the incoming sensory stimulus. These features tend to become increasingly specific and complex on ascending the hierarchy (Bond, 2004). For example the primary visual cortex, V1, is responsive to basic image features present in most stimuli (Hubel and Wiesel, 1959), whereas area MT is preferentially responsive to certain types of motion (Tootell et al., 1995) and the fusiform face area (FFA) is selective to faces (Kanwisher et al., 1997), suggesting MT and FFA are higher than V1 in the visual processing hierarchy (Grill-Spector and Malach, 2004). Similar patterns of increasing stimulus selectivity and complexity are found in auditory (Okada et al., 2010; Wessinger et al., 2001) and somatosensory (Iwamura, 1998) hierarchies.

The cortex also demonstrates hierarchical organization of regions based on structural characteristics, such as cortico-cortical connectivity. These data are most commonly derived through histological tracer studies in which dyes are injected to follow efferent axons to their target areas (Pandya and Sanides, 1973). The relative positions of two cortical areas within in a hierarchy are determined by the ratio of connection types, which can be feedforward (generally from lower to higher regions) or feedback (higher to lower). Feedforward axons
tend to project from more superficial cortical layers while feedback axons project from deeper layers (Markov et al., 2014; Pandya and Sanides, 1973). Tracer studies have revealed connection-based hierarchies in visual, somatosensory, motor (Felleman and Van Essen, 1991), auditory (Hackett et al., 1998) and prefrontal regions (Barbas, 1986).

At a microscopic scale, the cytoarchitecture of cortical areas mirrors their structural hierarchical organization. The degree of laminar differentiation is, for example, strongly related to a region’s connectivity and position within a sensory structural hierarchy (Barbas, 1986). Primary sensory regions have six clearly defined cytoarchitectural layers whereas, for higher-level areas, the laminar differentiation is less distinct. More generally it has been observed that primary sensory regions have increased neuronal density compared to other cortical areas (Collins et al., 2010; Scholtens et al., 2014).

While laminar structure and density might reflect the relative position of a region in a structural hierarchy (Scholtens et al., 2014), these patterns cannot yet be quantified using MRI. This limits the degree to which human structural and functional processing can be correlated in vivo. But it may be that other, more accessible parameters such as cortical thickness are related to cortical cytoarchitecture and can be used as an effective surrogate marker of laminar differentiation and by extension, structural hierarchy. For example, neuronal density and cortical thickness are generally inversely correlated (Cahalane et al., 2012; la Fougère et al., 2011), and both exhibit a similar rostral-caudal gradient across the cortex. These results tentatively support the hypothesis that a gradient of cortical thickness, from thinner primary sensory areas to thicker higher order areas, may be a useful biomarker of changes in cortical cytoarchitecture, structure and potentially functions of the hierarchy.

While there is some debate over the precise ordering of individual regions (Hilgetag et al., 1996), structural hierarchies do show consistent similarities to functional hierarchies. For example in the visual cortex, area MT is consistently higher than areas V1 and V2 (Felleman and Van Essen, 1991; Grill-Spector and Malach, 2004; Hilgetag et al., 1996; Markov et al., 2014); in the auditory cortex there is a matching progression from the auditory core, to the belt regions, and then to superior temporal gyrus and superior temporal sulcus (Hackett et al., 1998; Okada et al., 2010; Wessinger et al., 2001); and in the somatosensory cortex the hierarchy progresses caudally across the post-central gyrus, through BA (Brodmann Area) 3a, 3b, 1, 2 & 5 (Felleman and Van Essen, 1991; Iwamura, 1998). Structural and functional hierarchies may therefore describe the same principle of cortical organization. However several electrophysiological studies have demonstrated higher order characteristics in lower order regions (Hegdé and Van Essen, 2007; Lee, 2002), suggesting that sensory processing is not so easily simplified. Nevertheless if structural hierarchies index functional
hierarchies, then inter-individual variability in cortical cytoarchitecture and thickness may reflect differences in hierarchical function.

However empirical evidence that cortical thickness is a marker of structural hierarchy is lacking. A relationship between the thickness of a cortical area and its relative hierarchical position may be obscured by the mechanics of cortical gyrification, which cause gyri to be on average 20% thicker than sulci (Fischl and Dale, 2000; Van Essen and Maunsell, 1980). However these effects, though relatively under-explored, can be identified and accounted for using MR-based surface reconstruction approaches (Figure 2.3).

In summary, this study began by evaluating whether cortical thickness is a surrogate marker of laminar differentiation. Subsequently I investigated whether connectivity and functional hierarchies are organised in accordance with structural gradients. Structural MR images were acquired from macaque and human subjects. For each subject, thickness gradients were quantified for three separate hierarchies in each species, namely visual, somatosensory and auditory. Hierarchical level was identified in the macaque using standardized parcellation maps and tracer-derived hierarchies (Barbas, 1986; Felleman and Van Essen, 1991). With respect to the human dataset, given a lack of precise knowledge about the layout of structural hierarchies, geodesic distance from the primary sensory region was used as a proxy measure of hierarchical position, having validated this approach against macaque hierarchies. Geodesic distance also offers a means of analysis that obviates differences between the various hierarchical models that exist. Finally thickness was compared against a functionally derived visual hierarchy. Importantly, all measures of cortical thickness were adjusted to account for the local effects of folding.

2.3 Methods

2.3.1 MRI acquisition

Structural MRI data from one macaque (Macacca mulatta) and 83 human subjects were analyzed. The macaque data was acquired using a 3T Acheiva Phillips Scanner at Boston University School of Medicine. A T1-weighted 3DMPRAGE image (TR=7.09, TE=6, Flip=8) was obtained through the brain with 0.6 x 0.6 x 0.6 mm voxel size. The human subjects were scanned according to the Human Connectome Project Project (Milchenko and Marcus, 2013; Van Essen et al., 2013) protocol, using 3T Siemens Skyra Connectome Scanner at Washington University, St. Louis. A T1-weighted 3DMPRAGE image (TR=2400, TE=2.14, TI=1000, Flip=8, FOV=224x224) was obtained through the brain with 0.7 x 0.7 x 0.7 mm voxel size.
2.3 Methods

2.3.2 FreeSurfer reconstruction and cortical thickness

Cortical reconstructions were created using FreeSurfer software (Dale et al., 1999a; Fischl et al., 1999a,b). Cortical thickness was measured as the shortest distance between each vertex on the white matter surface and the pial surface (Fischl and Dale, 2000).

2.3.3 Parcellation and cortical hierarchies

The macaque surface reconstruction was registered to the F99 atlas using FreeSurfer & Caret software (Van Essen, 2002; Van Essen et al., 2001, 2012b). The F99 atlas contains several cortical parcellation schemes including FVE91 (Barbas, 1986; Felleman and Van Essen, 1991), used to outline somatosensory and visual cortical areas, PHT00 (Paxinos and Franklin, 2004) used for auditory areas and M132 (Markov et al., 2014) atlas used for comparison with laminar differentiation data.

Macaque visual and somatosensory hierarchies were taken from Felleman and Van Essen (Felleman and Van Essen, 1991); regions were delineated in the FVE91 parcellation scheme. Successive levels in the hierarchy were given numerical values, such that V1 has a hierarchical level of 1, V2 is level 2 etc. Several areas in the original visual hierarchy were omitted from this analysis: 7b, ER, 36, HC, FEF, 46, TF & TH. The first 4 of these are non-visual regions, TF and TH part of the parahippocampal cortex, structurally different to normal neocortex. Areas FEF and 46 are frontal regions and not part of the hypothesized continuous structural gradient. The somatosensory hierarchy was simplified from the somatomotor hierarchy (for discussion of motor hierarchy see Limitations).

The macaque auditory hierarchy is described by several studies (Barbas, 1986; Bond, 2004; Kaas et al., 1999; Rauschecker and Scott, 2009), which outline hierarchies with consistent characteristics, including anterolateral progression. For this study I took the hierarchy from Barbas (1986), a cytoarchitecture and tracer-defined hierarchy with 6 levels. Architectonic regions in the hierarchy are well described and each level is an aggregate of several areas in the PHT00 parcellation scheme. In this study the auditory hierarchy was manually outlined into these 6 cortical regions.

Human reconstructions were registered to the PALS-B12 atlas in Caret (Van Essen et al., 2012a) and into its component gyri and sulci in FreeSurfer (Destrieux et al., 2010). The human visual cortex was parcellated with a matching functional hierarchy, adapted from Grill-Spector & Malach (Grill-Spector and Malach, 2004) using regions found in the PALS-B12 atlas and V1 as identified by FreeSurfer (Hinds et al., 2008). Primary somatosensory cortex (BA 3b) (parcellated in FreeSurfer (Fischl et al., 2008)), post-central gyrus & sulcus, and superior parietal gyrus (Iwamura, 1998) were included as somatosensory regions. Primary
auditory cortex (Heschel’s gyrus/transverse temporal gyrus), superior temporal gyrus and transverse temporal sulcus were included as auditory regions (Rauschecker and Scott, 2009).

Due to an absence of matching functional hierarchy and parcellation schemes, geodesic distance from the primary sensory regions was used as a surrogate marker for hierarchical level for somatosensory and auditory hierarchies.

### 2.3.4 Geodesic distance

There is some debate over the precise details of cortical hierarchies (Barbas, 1986; Bond, 2004; Felleman and Van Essen, 1991; Hilgetag et al., 1996), but there is evidence that, within the visual hierarchy, hierarchical level increases with distance from the primary sensory region (Grill-Spector and Malach, 2004; Markov et al., 2014). For this reason geodesic distance from the primary sensory region to each randomly parcellated target region was used as a surrogate measure for hierarchical level. Geodesic distance was measured as the shortest path across the white matter surface between the vertices closest to the centre of each region (Figure 2.1). Distance was first validated against hierarchical level and cortical thickness in the all three macaque sensory hierarchies and human functional visual hierarchy, before being implemented as the sole measure of hierarchical level in human auditory and somatosensory hierarchies. The use of the geodesic distance as a marker for hierarchical level was necessarily speculative in human somatosensory and auditory cortex but, in the absence of an empirically validated means of identifying levels, I adopted these measures as a reasonable surrogate.

### 2.3.5 Individual variability and folding

Automated parcellation schemes are to some extent limited by individual variability. For example, there is a two-fold intersubject variability in the surface area of V1 (Andrews et al., 1997), and many borders are not consistently associated with large-scale morphological landmarks (Amunts et al., 2007b; Welker, 1990). To address the uncertainty over precise border locations, the cortex was randomly parcellated into 100 regions of approximately equal surface area; any region containing a border between different hierarchical levels was assigned the average value of the levels (Figure 2.2). By repeating random parcellation 10 times and averaging the thicknesses, the effect of folding (Figure 2.3) and the bias of each individual random parcellation scheme were minimized.
Fig. 2.1 Distance measures. Geodesic distance measures the shortest path between two points across the white matter (or pial) surface of the cortex. Euclidean distance is the shortest distance through 3-dimensional space. White matter tract distance approximates the length of an axon connecting two regions.
Fig. 2.2 Boundaries and parcellation. a) Addressing individual variability in atlas-defined boundaries. The dashed red line represents an atlas boundary between the orange area with hierarchical level 1 and the blue area with hierarchical level two. Randomly parcellated regions crossed by the red line are given the mean of the hierarchical levels. b) The random parcellation process is repeated 10 times, averaging cortical thickness values across parcellations to mitigate gyral-sulcal thickness differences.

2.3.6 Thickness validation

Because high cortical myelin content, as found in the primary sensory regions, can lead to underestimations in some MR measurements of cortical thickness (Glasser and Van Essen, 2011), I validated FreeSurfer cortical thickness in the human auditory cortex by comparing it to previously published histological data (Triarhou, 2007; von Economo and Koskinas, 1925). Average FreeSurfer values for human auditory cortex were 2.84mm and 2.88mm for the left and right hemispheres (SD=0.31mm), while histological measurement gives an estimate of 2.9mm. The close match with histological data is in keeping previous studies, which have validated FreeSurfer thickness measurements with histological comparison (Cardinale et al., 2014; Fischl and Dale, 2000).

2.3.7 Macaque histological data

Measurements of laminar differentiation in the macaque cortex were derived from an aggregate of multiple histological studies (Barbas, 1986; Barbas and Rempel-Clower, 1997; Dombrowski et al., 2001). Brain areas in the M132 atlas were ranked according to their cytoarchitectonic differentiation, taking into account features such as neuronal density and granularization of layer 4. For example the primary visual cortex with strong laminar dif-
Fig. 2.3 The effect of folding on cortical thickness. a) The red line shows a sample path across the cortical surface. Gyri are visibly thicker than their adjacent sulci. b) Unsmoothed MRI thickness values across one cortical hemisphere. Gyri are significantly thicker than sulci in a two-sample T-test (p<0.001). c) Hypothesised effect of folding on thickness values obscuring gradient. d) Actual data taken from a sample path proceeding anteriorly from V1 in one subject. Averaging across 10 random surface parcellations mitigates the effect of gyral-sulcal position.
ferentiation has laminar differentiation type 8, while areas with less clearly defined laminar structure have progressively rankings.

2.3.8 Statistics

Statistical analysis was carried out using Matlab & R (MATLAB, 2010; R Core Team, 2012). All relationships were tested using Spearman’s partial rank correlation controlling for the effect of hemisphere. Macaque data were also tested using a linear model, with individual hemispheres as covariates and human data were tested with a linear mixed effects model, with individual hemispheres as covariates and individual subjects included as a random effect.

2.4 Results

2.4.1 Macaque - cortical thickness and cytoarchitecture

Regional laminar differentiation data were compared with folding-corrected, MRI-derived cortical thickness values using Spearman’s partial rank correlation controlling for the effect of hemisphere. In agreement with the hypothesis, cortical thickness correlated negatively with laminar differentiation type ($r_s = -0.39, p<0.001$)(Figure 2.4).
Table 2.1 Results table for macaque and human sensory gradients.

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<th>Spearman’s partial rank correlations</th>
<th>Linear and mixed effects models</th>
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<td>Cortical thickness predicts hierarchy</td>
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<td>Human</td>
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<td>Cortical thickness and geodesic distance</td>
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<td>Auditory</td>
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Fig. 2.4 Macaque cortical thickness and cytoarchitecture. MRI thickness - corrected for folding - against laminar differentiation type ($r_s=-0.39$, $p<0.001$). Laminar differentiation type is a cytoarchitectural ranking scale, with 6-layered primary sensory cortex having type 8, while less well differentiated cortical regions are given progressively lower rankings (Barbas, 1986; Barbas and Rempel-Clower, 1997; Dombrowski et al., 2001).

2.5 Cortical thickness, hierarchical level and geodesic distance

Macaque data were analysed using Spearman’s partial rank correlation and a linear model, controlling for the effect of hemisphere (see Table 2.1). In agreement with the hypothesis, hierarchical level was significantly correlated with and predicted by cortical thickness in visual, somatosensory and auditory cortices, where hierarchical level increased with thickness (Figures 2.5, 2.6 & 2.7). In the somatosensory cortex the correlation was not as strong but was still significant.
2.5 Cortical thickness, hierarchical level and geodesic distance

Fig. 2.5 Visual cortex: cortical thickness, geodesic distance and hierarchical level for a single macaque and human. Left column: folding-corrected cortical thickness (mm) for the visual cortex with greyscale lines of iso-geodesic distance (mm) from the primary visual cortex (V1). Middle column: continuous measure of geodesic distance from V1. Right column: structural hierarchical level of visual regions based on axonal tracer studies in the macaque (Felleman and Van Essen, 1991) and functional hierarchical level of visual regions based on fMRI in the humans (Grill-Spector and Malach, 2004). Correlations between cortical thickness, geodesic distance and hierarchical level are highly significant (p<0.001). Data overlaid on inflated left hemispheres, lateral and medial views. Rostral (R), caudal (C).
Fig. 2.6 Somatosensory and auditory cortices: cortical thickness, geodesic distance and hierarchical level for a single macaque and human. Left column: folding-corrected cortical thickness (mm) with greyscale lines of iso-geodesic distance (mm) from the primary sensory cortex (S1 or A1). Middle column: continuous measure of geodesic distance from S1/A1. Right column: structural hierarchical level of somatosensory and auditory regions based on axonal tracer studies and cytoarchitecture in the macaque (Barbas, 1986; Felleman and Van Essen, 1991). Matching hierarchies and cortical parcellations were not available for humans. Correlations between cortical thickness, geodesic distance and hierarchical level are highly significant. Data overlaid on inflated left hemispheres, lateral views. Rostral (R), caudal (C).
Fig. 2.7 Macaque structural hierarchies. Graphs showing that cortical thickness correlates with structural hierarchy and geodesic distance from the primary sensory area in the macaque (see Table 2.1 for statistical results). Blue lines and points show that as hierarchical level increases cortical thickness (mm) also increases, for visual, somatosensory and auditory hierarchies. Red lines and points show geodesic distance (mm) - the putative surrogate of hierarchical level - increasing with cortical thickness for all three sensory hierarchies. Solid lines and filled circles show left hemisphere, dashed lines and hollow circles show right hemisphere.
Hierarchical level was significantly correlated with and predicted by geodesic distance in all three hierarchies. This result is important for human somatosensory and auditory hierarchies, where there was no available parcellation for literature-based hierarchy.

Finally, geodesic distance from the primary sensory region was also predicted by cortical thickness in the visual, somatosensory and auditory cortices. Again the relationship in the somatosensory cortex was not as strong, but the correlation was still significant.

Human data were analysed using Spearman’s partial rank correlation and a linear mixed effects model, controlling for the effect of hemisphere and the random effects of individuals (see Table). In agreement with the macaque data and the original hypothesis geodesic distance was significantly predicted by cortical thickness in the visual, somatosensory and auditory cortices (Figures 2.5, 2.6, & 2.8). For somatosensory and auditory cortices the correlations were not as strong but were still significant (Figure 2.8).

For the visual cortex, functional hierarchical level was significantly predicted by cortical thickness and geodesic distance from the primary visual cortex (Figure 2.9 & 2.5).

2.6 Discussion

In this investigation I tested the hypothesis that cortical thickness may be a useful marker of cytoarchitecture - one that could be used to identify gradients in sensory structural hierarchies. The results demonstrate a strong relationship between cortical thickness, laminar differentiation and estimated hierarchical position for visual, auditory and somatosensory hierarchies. Furthermore, these data are compatible with a close coordination of cortical structure and function. Critically, the findings overall suggest that patterns of cortical thickness, which are readily measurable in vivo with structural neuroimaging, can be interpreted meaningfully in terms of underlying patterns that relate directly to cortical function. They offer a framework for analysing and interpreting cortical thickness measures in health and disease.

2.6.1 Interpreting cortical thickness

In the macaque, cortical thickness - corrected for effect of folding - was negatively correlated with laminar differentiation. While previous studies have shown a relationship between thickness and neuronal density (Cahalane et al., 2012; Collins et al., 2010; la Fougère et al., 2011), an inverse correlation with laminar differentiation has not previously been demonstrated. Moreover, throughout the cortex there are a number of inter-dependent and predictable relationships between various cytoarchitectural properties. For example neuronal density can be reliably related to intracortical connectivity, such that lower neuronal density
2.6 Discussion

Fig. 2.8 Human structural hierarchies. Graphs show that cortical thickness increases with geodesic distance from the primary sensory area in human sensory cortices (See Table for statistical results). Geodesic distance is used as a surrogate marker of structural hierarchical level. Upper graphs: data from all human subjects. Points represent mean thickness value for a random sample region across 83 subjects; error bars represent population standard deviation. Lines show linear models with grey 95% confidence band for population trend. Solid lines and filled circles show left hemisphere, dashed lines and hollow circles show right hemisphere. Lower graphs: trend lines of individual data from 10 subjects. These plots show the consistent structural gradients across individuals and also a degree inter-individual structural variability. This variability may be of interest in healthy development and psychopathology.
Fig. 2.9 Human functional hierarchy. Cortical thickness (mm) increases with fMRI-derived functional hierarchical level (Grill-Spector and Malach, 2004) in humans (See Table for statistical results). Points represent mean thickness value for a random sample region across 83 subjects; error bars represent population standard deviation. Lines show linear model with grey 95% confidence band for population trend. Solid lines and filled circles show left hemisphere, dashed lines and hollow circles show right hemisphere.
is associated with increased dendritic arborization and density of synapses (Cullen et al., 2010; Elston, 2003; Schüz and Palm, 1989). The inter-relationship between neuronal density, laminar differentiation and cortical thickness may be useful in considering thickness changes in studies of structural MRI.

### 2.6.2 Cortical thickness, structural hierarchies and function

Cortical hierarchies have previously been characterized by laminar differentiation and by layer-specific cortico-cortical connections. Here I have shown that cortical thickness may be a further indicator of hierarchical level. Each of these measures brings its own advantages and functional implications. Firstly, layer-specific interregional connections are considered functionally as indexing feed-forward (signals going from lower to higher regions) and feedback connections (higher to lower signalling). Hierarchical connectivity patterns such as these have previously been characterized using axonal tracer studies indicating that: i) feedforward connections predominantly originate in upper cortical layers and terminate in the lower layers of a target region and ii) feedback connections project from lower and terminating in upper layers (Markov et al., 2014; Pandya and Sanides, 1973).

Inter-regional feedforward and feedback connectivity has been related to integration of sensory information in the context of increasingly influential predictive coding models (Bastos et al., 2012). Within such models, the balance of feedforward/feedback connections determines functional organization. Thus, the patterns of laminar-specific connectivity have important implications for how structure may govern and shape function.

A second measure of structural hierarchy is cytoarchitectural. The pattern of feedforward/feedback innervation is closely linked to laminar differentiation (Barbas, 1986; Barbas and Rempel-Clower, 1997), neuronal density and even dendritic tree size and spine density (Scholtens et al., 2014). Furthermore some of the changes in neuronal density are layer specific, where caudal cortical regions have increased density in supragranular layers (Charvet et al., 2013), which give rise to feedforward axons (Markov et al., 2014; Pandya and Sanides, 1973). Rather than describing an identical, repeated neuronal microcircuit, these measures emphasise systematic variation in intra-cortical connectivity throughout the hierarchies.

This study shows that cortical thickness follows a similar gradient to hierarchies of connectivity and cytoarchitecture, and might therefore offer a third, related marker of structural hierarchy. Critically, unlike laminar connectivity and cytoarchitectural patterns, thickness gradients are accessible to standard neuroimaging tools. Thus, the identification of this potential marker of non-invasive, in vivo marker of cytoarchitectural trends offers a framework for interpreting cortical thickness patterns which are frequently reported, though poorly understood, in health and disease. Moreover, the observation of a gradient of cortical thickness
Cortical thickness gradients in structural hierarchies

within the fMRI-based human visual hierarchy (Grill-Spector and Malach, 2004) suggests a, hitherto unestablished, mapping between structural and functional hierarchies. Although such a relationship remains speculative, the results support the idea that structural regularities have a direct functional correlate.

2.6.3 Cortical hierarchies in development and disease

Laminar connectivity, cytoarchitecture and thickness change over the course of development. There is little change in the number of feedforward connections postnatally (Batardière et al., 2002), while both feedback connections, synaptic density and cortical thickness increase postnatally up to a peak and then decrease (Barone et al., 1995; Goldman-Rakic, 1987; Price et al., 2006; Shaw et al., 2008). Based on this rationale, I hypothesize developmental changes in thickness gradients mark changes in cortical cytoarchitecture and connectivity that support higher level sensory processing.

During childhood, thickness increases at a differential rate across the cortex, with various regions achieving peak thickness at different time points between the ages of 7 and 11 (Gogtay et al., 2004; Raznahan et al., 2011; Shaw et al., 2008). Of note, hierarchical gradients develop in a gradual and non-uniform way, with primary sensory areas reaching peak thickness before secondary and higher association areas (Gogtay et al., 2004). It has been observed that the timing of thickness peaks is correlated with functional development. For example, in the visual system the occipital cortex peaks at roughly the same age that children develop letter acuity and global motion detection (Lewis and Maurer, 2005). These observations suggest that by quantifying the thickness gradient in a sensory structural hierarchy, it may be possible to more accurately interpret the stages of development.

More generally a gradient-based approach to structural analysis obviates another important limitation of such analysis, namely the high inter-individual variation in the pattern of morphology and cytoarchitecture across the cortex (Rajkowska and Goldman-Rakic, 1995). By assessing gradient differences across structural hierarchies as opposed to individual regions, the ambiguity of regional identity is somewhat mitigated. Moreover, larger-scale regional analyses might be informative in conditions of atypical neurodevelopment such as autism and schizophrenia, which are associated with complex distributed changes across the cortex and are not reducible to a single morphological or functional abnormality (Chung et al., 2005; Ecker et al., 2013; Fletcher and Frith, 2009; Kuperberg et al., 2003; Rimol et al., 2012; Ronan et al., 2012).
2.6 Discussion

2.6.4 Cortical thickness and folding

It is important to note that observations of the relationships between morphology, cytoarchitecture and functional organization were made having taken steps to account for the effect of folding, which causes gyri to be significantly thicker than sulci (Fischl and Dale, 2000) (Figure 2.3). This folding effect may obscure the large-scale thickness gradient of interest and therefore its effect was minimized through the random parcellation-based smoothing (Figure 2.2 & 2.3). In fact, this approach is almost equivalent to surface-based Gaussian kernel smoothing.

It is noteworthy that, in general, individual differences in cortical folding patterns could obscure or introduce group cortical thickness differences. Smoothing at a study-appropriate scale may help to mitigate these folding effects. Indeed this might underpin increases in sensitivity to group thickness differences observed when applying surface-based Gaussian smoothing kernels at 30mm FWHM (Lerch et al., 2005).

2.6.5 Limitations

A number of limitations attend this study. First, there is on-going debate over the precise organization of structural hierarchies and it can be difficult to assert that any given sub-region belongs to a single specific level within a hierarchy (Hilgetag et al., 1996; Markov et al., 2014). Moreover, it is simplistic to suppose that structural hierarchies comprehensively and exhaustively describe functional organization and information flow, and there are several possible measures of hierarchical function (Bond, 2004). I aimed to mitigate these concerns by demonstrating only the general trend of cortical thickness in structural hierarchies. Having done this in those hierarchies about which previous literature was clearest, a complementary analysis using geodesic distance as a surrogate marker of hierarchical levels was used to further validate the approach. Whilst this approach overlooks some of the subtleties of structural hierarchies, I nevertheless believe the results persuasively demonstrate a variability in cortical thickness that closely correlates with previously established markers of structural hierarchy and certain measures of functional hierarchy.

In this study I also explored the validity of the measure of cortical thickness, given the confounding effect of cortical myelin content (see Methods). High cortical myelin content, as found in the primary sensory regions, can lead to underestimations in some MR measurements of cortical thickness (Glasser and Van Essen, 2011). However the analysis of the auditory cortical thickness indicated a close match between FreeSurfer cortical thickness measurement and histological data, which is in keeping previous validation studies (Cardinale et al., 2014; Fischl and Dale, 2000).
Some of the results in the somatosensory and auditory cortices, while significant, are not particularly strong ($r_s < 0.35$). This may in part be due to the hierarchies being relatively small, both in terms of the number of hierarchical levels and the area over which they extend. Nevertheless the results remain significant suggesting that these structural principles do still apply in these sensory areas.

This analysis was confined to the cortical thickness gradients in sensory processing hierarchies, such that these results may not be generalizable to motor, frontal and prefrontal cortices. While there is evidence for the existence of motor and frontal hierarchies, both structural (Barbas, 1986; Felleman and Van Essen, 1991; Goulas et al., 2014), and functional (Badre, 2008), several considerations prevent the simple extension of the hypothesis to include these pathways for this experiment. First, the structure of frontal and prefrontal cortices is far more variable (Dombrowski et al., 2001). Unlike sensory cortices, primary motor and premotor areas have an agranular cytoarchitecture and are particularly thick (Brodmann, 1909), despite having clear laminar differentiation (Barbas, 1986). Similarly, the frontal cortex does not follow the same inverse relationship between neuronal density and cortical thickness seen in all other cortical regions (la Fougère et al., 2011). Moreover, the direction of information flow is reversed, with signals predominantly traveling caudally from frontal 'abstract' areas towards the primary motor cortex (Badre and D’Esposito, 2009). Therefore while cortical thickness gradients may be of interest in frontal-motor hierarchies, they are unlikely to follow the pattern seen in sensory cortices.

Finally, it should be noted that this experiment used data from a single macaque as a proof-of-concept analysis, capitalizing on the high degree of specificity with which the functional organization is known in this species. Although this necessarily limits the generalizability of this analysis, the closely matched findings in the 83 human subjects support the hypothesis that gradients of cortical thickness co-vary with hierarchical level, and suggest that this may be a general principle of brain organization and structure.

2.6.6 Conclusions

These findings offer a new framework for the interpreting cortical thickness measured using structural MRI. Cortical thickness gradients are significantly correlated with structural hierarchical level of sensory processing hierarchies in macaque and human subjects. Multiple lines of evidence suggest that this relationship is further characterized by predictable changes in cortical cytoarchitecture and connectivity. The results also suggest a close coupling between cortical structure and functional demand, such that higher-order visual areas tend to be thicker. Analysis of these gradients may advance our understanding of the structure-function relationship in normal and atypical neurodevelopment.
2.6.7 Acknowledgements

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Chapter 3

Multiple markers of cortical morphology reveal evidence of supragranular thinning in schizophrenia

3.1 Abstract

*In vivo* structural neuroimaging can reliably identify changes to cortical morphology and its regional variation but cannot yet relate these changes to specific cortical layers. I propose, however, that by synthesizing principles of cortical organization, including relative contributions of different layers to sulcal and gyral thickness, regional patterns of variation in thickness of different layers across the cortical sheet and profiles of layer variation across functional hierarchies, it is possible to develop indirect morphological measures as markers of more specific cytoarchitectural changes. Four indirect measures sensitive to changes specifically occurring in supragranular cortical layers were developed to test the hypothesis that supragranular layers are disproportionately affected in schizophrenia. The findings from the four different measures converge to indicate a predominance of supragranular thinning in schizophrenia, independent of medication and illness duration. These indirect measures offer novel ways of identifying layer-specific cortical changes, offering complementary *in vivo* observations to existing post-mortem studies.

3.2 Introduction

The cerebral cortex has a predictably varying laminar structure (Brodmann, 1909). Individual layers exhibit differing histological composition, regional distributions (von Economo and
Multiple markers of cortical morphology reveal evidence of supragranular thinning in schizophrenia (Parker, 1929), developmental trajectories (Conel, 1939), physiology (Douglas and Martin, 1991) and hypothesized functional roles (Bastos et al., 2012). The question arises whether a subtle variation in these components could contribute to the spectrum of psychiatric syndromes including schizophrenia (Harrison, 1999). However, direct identification of these putative changes is necessarily limited to post mortem analysis given that structural MRI methods currently cannot resolve individual cortical layers in vivo. This problem was addressed indirectly by capitalizing on principles of structural brain organization that potentially differentiate between alterations in the infragranular (V-VI) and supragranular (I-III) layers of the cortex. Specifically, four measures were identified that are relatively specific to supragranular change and used these measures to evaluate a previously acquired dataset (Ronan et al., 2012) from people with schizophrenia, in order to determine whether measurable neuroanatomical changes favoured the prediction that cortical pathology in schizophrenia is predominant in supragranular layers. The four measures are explained below.

First, the thicknesses of supra- and infragranular layers are consistently different between the crowns of gyri (where infragranular layers are thicker) and the depths of sulci (where supragranular layers are thicker) (Bok, 1929; Hilgetag and Barbas, 2006; von Economo and Parker, 1929; Welker, 1990). This is a product of deformation of the cortex under folding, such that layers that are on the outside of a fold (lower layers in sulci and upper layers in gyri) are stretched and thinner, while layers on the inside of folds are compressed and thicker (Hilgetag and Barbas, 2006). For example, in the prefrontal cortex, the supragranular layers make up 70% of the thickness of a sulcal fundus but only 49% of the adjacent gyral crown (von Economo and Parker, 1929). Thus, despite sulci being generally thinner than gyri (Fischl and Dale, 2000; von Economo and Parker, 1929) supragranular layers have an increased relative and absolute thickness in sulci. It follows from these clear folding-related differences in sulci and gyri that supragranular thinning will affect sulci disproportionately. This was the first hypothesis tested here. Specifically, if neuropathology in schizophrenia results in a disproportionate reduction in supragranular layer thickness, one would expect to measure disproportionate thinning of sulci, particularly in regions where these layers are more prominent. This is a general principle that may be applied to the whole brain or to individual lobes and brain regions.

Second, turning to small-scale (but quantifiable) measures of cortical morphometry in the form of intrinsic curvature (Ronan et al., 2011), I predict that these will also vary with supragranular layer changes (Ronan et al., 2012). Specifically, changes observed superficially (on the pial surface) could feasibly be related to thinning of superficial layers while those observed on the white matter surface could more feasibly be related to thinning in deeper
3.2 Introduction

layers. Moreover, in line with the reasoning outlined above, these changes are expected to be more prominent in sulci than gyri.

Third, it is known that cytoarchitecture is not uniform across the healthy human cortex (Brodmann, 1909) – and that layer thicknesses exhibit significant and consistent variability (Charvet et al., 2013; von Economo and Parker, 1929). One clear variation in regional patterns of supragranular thickness can be seen across dorsolateral prefrontal cortex/BA46 (where it tends to be thicker) and the anterior cingulate/BA24 cortex where it is particularly thin. I therefore hypothesized that supragranular thickness alterations in pathological cases may be more prominent in DLPFC (where these layers are thicker) than in anterior cingulate cortex for example where these layers are thinner. An extension of this hypothesis is that the pattern of sulcal-specific thinning should follow supragranular layer thicknesses in healthy individuals (von Economo and Parker, 1929).

Finally, as well as gyral-sulcal differences, gradients of supragranular (I-III/IV) layer thickness exist within sensory hierarchies. In particular, sensory hierarchies have a gradient of absolute cortical thickness, with thinner primary sensory regions and progressively thicker higher cortical regions (Wagstyl et al., 2015). Supragranular layers are the origin of feed-forward connections, communicating incoming sensory information. They are relatively thicker in these lower sensory regions (Charvet et al., 2013; von Economo and Parker, 1929). By contrast infragranular, feedback layers (V-VI) are more prominent in higher sensory and frontal cortical areas. Therefore a loss of supragranular thickness should result in a relatively steeper gradient of cortical thickness across the structural hierarchy of sensory systems (i.e. preferential reduction of cortical thickness in lower sensory regions).

In summary, I aimed to capitalize on basic biological observations of the natural variation in supragranular thickness in order to relate alterations in MR-based measurements of cortical thickness and curvature to underlying supragranular changes. In order to demonstrate the applicability of these measures, they were applied in a schizophrenia case-control cohort. Accumulating evidence from several approaches has indicated that there are alterations in the supragranular layer in schizophrenia. This includes deficits in small interneurons (Benes et al., 1991), reduced density of calbindin cells in layer II (Chance et al., 2005), as well as changes in neurotransmitters, receptors (Curley and Lewis, 2012), pyramidal cell density and morphology (Cullen et al., 2006; Garey et al., 1998) and mRNA expression (Joshi et al., 2015) in layers II and III. In addition supragranular thinning has been measured in the dorsolateral prefrontal cortex (BA46) (Selemon et al., 1998) although the extent of these changes across the cortex has not been established. However these measures of supragranular change are not accessible \textit{in vivo}. It is extremely useful therefore to develop surrogate markers of these
Multiple markers of cortical morphology reveal evidence of supragranular thinning in schizophrenia.

Fig. 3.1 Morphological effect of disproportionate supragranular thinning (I-III, green/blue, infragranular V-VI light grey). Decreased dendrites and synapses in schizophrenia can result in thinner supragranular layers, which may in turn be reflected in changes to large-scale cortical morphology. In particular, supragranular layers are thicker in sulci than in gyri, thus pruning will lead to disproportionately reduced cortical thickness in sulci compared to gyri. Similarly, pruning of the supragranular layers will affect the curvature of the pial surface more than the curvature of the boundary between grey and white matter, again disproportionately more in sulci than gyri.
changes since they offer a possibility for going beyond the resolution of existing in vivo neuroimaging techniques in studying neuropsychiatric conditions.

The main objective was therefore to quantify pattern-specific cortical thinning measured on structural T1-weighted MRI scans of patients with schizophrenia. I predicted that the cortex in schizophrenia would not only be thinner in line with previous MR-based and neuropathological studies, but that disproportionate changes to the upper cortical layers would, on the basis of the above principles, cause thinning to be disproportionate in sulci, (which have a greater supragranular thickness) and lead to abnormal curvature of the pial, but not the white matter surfaces, particularly in sulci. In addition, the principles lead to the hypothesis that disproportionate sulcal thinning would be greater in regions with thicker supragranular layers such as BA46 but not in anterior cingulate cortex. Finally I predicted that the gradient of thickness in the visual hierarchy would be steeper in patients with schizophrenia. These four measures taken together may be adopted as macroscale surrogate markers of changes not currently accessible with current MRI measures.

3.3 Methods

3.3.1 Subjects

Forty-six patients (36 males) (33.2±9 years) were recruited by collaborating psychiatrists from Oxfordshire and Berkshire Mental Healthcare Trusts, and with the guidance of the Oxford and Berkshire Psychiatric Research Ethics Committees, UK. Diagnosis was confirmed using the Structural Clinical Interview for DSM-IV Disorders (First et al., 1997). Forty-four controls (32 males) were also recruited (30.4±8 years). There were no statistically significant differences in age between patients and controls. Medication and dose were available for 31 patients from which were derived chlorpromazine equivalent doses. Duration of illness was calculated for 32 patients.

3.3.2 MRI acquisition

Structural MRI data were acquired using a 1.5 T Sonata MR imaging system (Siemens Medical Systems Limited, Erlangen, Germany) with a standard quadrature head coil and maximum 40 mT m$^{-1}$ gradient capability at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR). Whole brain T1-weighted images were acquired with a FLASH sequence using the following parameters: coronal orientation, image matrix = 256 × 256, with 1×1 mm$^2$ in-plane resolution, 208 slices of slice thickness 1 mm, TE=5.6 ms, TR=12 ms, and flip angle α=19°.
Multiple markers of cortical morphology reveal evidence of supragranular thinning in schizophrenia.

3.3.3 Cortical reconstruction and analysis

Cortical reconstructions were generated using the software FreeSurfer 5.2 (Dale et al., 1999b; Fischl and Dale, 2000; Fischl et al., 1999a). In brief, raw image data voxels were resampled to voxels of side 1 mm$^3$. The data were then normalized for intensity, RF-bias field inhomogeneities were modeled and removed, followed by skull-stripping. The cerebral white matter was subsequently identified after which the hemispheres were separated, tessellated and deformed to produce an accurate and smooth representation of the gray–white interface. These surface reconstruction processes were conducted in native space. To correct for minor inaccuracies, the reconstructions were manually edited. Eight scans (3 patients, 5 controls) were omitted from further analyses due to large errors or artifacts. Mean curvature was measured to divide the cortex into gyri and sulci. Gyri have a negative mean curvature; sulci have a positive mean curvature.

3.3.4 Morphometric measurements of supragranular layer thickness changes

Four distinct morphometric markers of supragranular thickness changes were developed, namely:

Whole brain gyral-sulcal thickness differences

Cortical thickness was measured as the shortest distance between each vertex on the white matter surface and the pial surface (Fischl and Dale, 2000). Mean gyral thickness and mean sulcal thickness was calculated for each individual, as was the difference between these two measures. The ratio between total gyral and sulcal surface area was also calculated to test for systematic changes in cortical surface classification.

Whole brain gyral-sulcal intrinsic curvature differences

Intrinsic or Gaussian curvature was calculated for each vertex on the cortex on the white matter and pial surface reconstructions as the product of the principal curvatures (Ronan et al., 2011, 2012). Mean, modulus of intrinsic curvature was calculated for gyral and sulcal cortex at the white matter and pial surfaces, along with the difference between gyral and sulcal measurements.
Regional specific pattern

A local measure of gyral-sulcal thickness differences (GSD) was created as a normalized difference between mean gyral and sulcal cortical thickness within a 25mm radius of each vertex i on an inflated cortical surface.

\[
GSD_i = \frac{G_m - S_m}{G_m + S_m}
\]  

(3.1)

The value of this measure increases when gyral-sulcal thickness differences increase. Thus an increase in GSD is taken as a measure of the extent to which thinning is sulcal-specific. 25mm was chosen as the disc radius to balance local specificity and capturing sufficient gyral and sulcal cortex, independent of central vertex location (Schaer et al., 2008). Figure 3.2 shows the effect of varying disc radius on the relative areas of gyral and sulcal cortex captured by the disc. Per-vertex GSD was registered from individuals to an average surface and comparison was carried out between patients and controls, controlling for differences in white matter total surface area. Total brain surface area is related to other morphometric measures like cortical thickness and therefore was taken into account in the regression model (Im et al., 2008). Furthermore, in order to assess the specificity of the gyral-sulcal derived markers of supragranular thinning, the regional measure of sulcal-specific thinning was compared to previously reported post mortem findings. Thickness measurements for cortical regions were taken from von Economo 1929. Where explicit measurement for a
layer was omitted, approximate layer thicknesses were inferred based on textual description, annotated figures and comparison with measurements from the remaining 5 layers. These values were compared to regional measures of sulcal-specific thinning in schizophrenia using the population-average, landmark- and surface-based (PALS) atlases of Brodmann areas (Van Essen, 2005; Van Essen and Dierker, 2007). Von Economo regions were identified based on the Brodmann atlas and reference tables (Zilles et al., 2015b).

Cortical thickness gradient in visual hierarchy

Cortical thickness gradients across the visual hierarchy were calculated for all subjects (Wagstyl et al., 2015). Briefly, visual regions were parcellated on individual subjects according to the PALS visuotopic atlas (Van Essen, 2005; Van Essen and Dierker, 2007). These regions were given estimates of hierarchical level derived from functional studies (Grill-Spector and Malach, 2004). The gradient of regional cortical thickness against visual hierarchical position was calculated using a linear model. Gradients were then compared between patients and controls, accounting for hemispheric differences.

3.3.5 Statistical analysis

Statistical analyses of the data were carried out using Matlab & R (MATLAB, 2010; R Core Team, 2012). Patient-control differences were calculated for each of these measures using a linear mixed effects model (LME), controlling for hemispheric differences, total surface area, age, gender and the random effect of individual. The effect of medication and illness duration on measures 1, 2 and 4 were calculated within the patient group with an LME accounting for hemispheric differences, total surface area, gender and the random effect of individual.

3.4 Results

3.4.1 Whole brain gyral-sulcal thickness differences

In line with neuropathology and previous neuroimaging studies, cortical thickness was significantly decreased in patients with schizophrenia in both gyri (B=-0.21, t=-6.20, p<0.0001) and sulci (B=-0.29, t=-7.12, p<0.0001)((Figure 3.3(a)(i), see Figure 3.4 for vertex-wise cortical thickness differences). The difference between group’s mean gyral and sulcal thickness was calculated as the interaction between group (patient vs control) and morphology (gyral vs sulcal). Thinning was disproportionately in the sulci of patients with schizophrenia (B=-0.08, t=-6.87, p<0.0001) (Figure 3.3(a)(ii)). This is in line with the hypothesis that
supragranular pathology would be differentially expressed in sulci, owing to the relatively increased supragranular thickness in these regions. There were no significant differences in the ratio of gyral/sulcal cortical area (p=0.39), thus increased gyral-sulcal thickness difference was not caused by systematic misclassification of the cortical surface. There was no significant effect of medication (p=0.12) or illness duration (p=0.14) on gyral-sulcal thickness differences within the patient group.

Fig. 3.3 (a) (i) Cortical thickness is decreased in both gyri and sulci in schizophrenia (p<0.0001). (ii) Supragranular layers are thicker in sulci so that supragranular pathology in schizophrenia leads to a disproportionate decrease in the thickness of the cortex in sulci compared to gyri (p<0.0001). (b) (i) Intrinsic curvature at the pial surface is decreased in both gyri and sulci in schizophrenia (p<0.0001). Consistent with a predominantly upper cortical layer change, there was no difference in intrinsic curvature at the white matter surface. (ii) For the same reason as in (a) (ii) above, sulcal intrinsic curvature is disproportionately decreased relative to gyral curvature in schizophrenia (p<0.0001).

3.4.2 Intrinsic curvature

Consistent with upper cortical layer changes driven by supragranular pathology, there was a significant reduction in pial surface intrinsic curvature of both gyri (B=-0.017, t=-5.59, p<0.0001) and sulci (B=-0.040, t=-6.97, p<0.0001) (Figure 3.3(b)(i)), but no change in curvature of the white matter surface intrinsic curvature of gyri (B=0.001, t=0.29, p=0.77)
Multiple markers of cortical morphology reveal evidence of supragranular thinning in schizophrenia or sulci ($B=0.001, t=0.44, p=0.66$). Moreover, given the increased prominence of upper cortical layers in sulci, the sulcal curvature was disproportionately decreased, relative to gyral curvature in subjects with schizophrenia ($B=0.023, t=7.21, p<0.0001$)(Figure 3.3(b)(ii)). There was no effect of medication ($p=0.92$) or illness duration ($p=0.22$) on gyral-sulcal curvature differences within the patient group.

### 3.4.3 Regional Specific Pattern

In line with the hypotheses gyral-sulcal thickness differences (GSD) were non-uniformly increased in schizophrenia. Areas of the dorsolateral prefrontal cortex (DLPFC/BA46), temporal and parietal cortex exhibit significantly increased, gyral-sulcal differences (Figure 3.5(a)). The anterior cingulate cortex showed decreased GSD in line with the neuropathological studies finding no measurable supragranular change (Benes et al., 2001). These findings in the DLPFC and anterior cingulate cortex are consistent with previously published neuropathology studies measuring layer thicknesses in schizophrenia (Figure 3.5(b)). The pattern of changes in gyral-sulcal differences in schizophrenia was related to neuropathological layer II thickness measurements taken from healthy individuals ($B=-4.17, t=-2.11, p<0.05$). This was not true of other cortical layers (Figure 3.6), suggesting that gyral-sulcal thickness difference was uniquely sensitive to layer II changes. The pattern of changes in schizophrenia further suggested that sulcal-specific thinning was more significant in regions with a normally thicker layer II.

### 3.4.4 Cortical Thickness Gradient in Visual Hierarchy

Cortical thickness was strongly correlated with regional estimates of hierarchical level in both patients and controls (Figure 3.7(a)). However, the hierarchy-thickness gradient was significantly steeper in patients with schizophrenia than in healthy controls ($B=0.0064, t=6.24, p<0.0001$)(Figure 3.7(b)). This is consistent with disproportionate supragranular thinning, as these layers are more prominent lower in a sensory hierarchy. There was no effect of medication ($p=0.86$) or illness duration ($p=0.91$) on gradients of cortical thickness within the patient group.

### 3.5 Discussion

*In vivo* structural MRI measures cannot currently resolve cortical layers and therefore our ability to interpret morphological changes in terms of underlying pathological processes affecting different cortical layers is limited. In this paper I sought to develop surrogate markers
Fig. 3.4 Statistical maps showing cortical thickness differences, between controls and patients. Thickness values were smoothed at 10mm, and the z-scores are thresholded at p<0.01 (FDR-corrected). Consistent with previous imaging studies of schizophrenia, there are widespread reductions in cortical thickness, including in frontal, temporal, parietal and occipital lobes.
Multiple markers of cortical morphology reveal evidence of supragranular thinning in schizophrenia.

Fig. 3.5 Per-vertex comparison of gyral-sulcal thickness differences (GSD). (a) Local gyral-sulcal thickness differences are regionally increased particularly in dorsolateral prefrontal cortex (DLPFC/BA46), superior temporal gyrus, inferior temporal gyrus and inferior parietal gyrus. (b) The regional pattern of sulcal-specific thinning is consistent with neuropathology studies of schizophrenia, which have identified layer II thinning in BA46 (Selemon et al., 1998), but not BA9, BA44 (Selemon et al., 2003) or anterior cingulate cortex (Benes et al., 2001).
Fig. 3.6 (a) Regional gyral-sulcal cortical thickness difference (GSD) compared with histological measurements of thickness for each cortical layer and their combined total cortical thickness, taken from von Economo 1929. (b) Only layer II thickness is significantly correlated (p<0.05) with the regional measure of sulcal-specific thinning in schizophrenia.
Multiple markers of cortical morphology reveal evidence of supragranular thinning in schizophrenia.

Fig. 3.7 (a) Cortical thickness increases with visual hierarchical level in both controls and patients with schizophrenia. Visual regions are listed below their assigned hierarchical level. (b) The gradient of thickness against hierarchical level is steeper in patients with schizophrenia (p<0.0001). This is consistent with supragranular thinning and has implications for the balance of feedforward/feedback connectivity in sensory regions.
of cortical structure that are sensitive to supragranular layer-specific changes. These markers are based on predictable patterns of cytoarchitecture elucidated by post mortem studies, which have indicated that laminar thicknesses have a close relationship with macroscale structure (Hilgetag and Barbas, 2006; von Economo and Parker, 1929). I was therefore able to identify four morphological changes consistent with supragranular thinning, namely (i) disproportionate gyral-sulcal thinning, (ii) disproportionate alterations in gyral-sulcal pial surface intrinsic curvature, (iii) a region specific pattern of sulcal thinning and (iv) a steeper gradient of thickness across the visual hierarchy. These inter-linked markers were tested on existing data from people with schizophrenia and matched controls. Taken together the markers indicated that supragranular changes are present in vivo in patients with schizophrenia and support the evidence from functional and histological modalities that some of the deficits associated with schizophrenia may have their origin in upper cortical layer pathology (Curley and Lewis, 2012; Fogelson et al., 2014; Harrison, 1999). Moreover these measures can be applied more generally as in vivo structural markers of layer specific change.

In this experiment, schizophrenia was adopted as a proof-of-concept case predicated on evidence from a wide range of neuropathology studies that identify changes to the supragranular cortical layers in the disease. In each case, the results were in agreement with pathological evidence. For example markers for supragranular thinning were found in the dorsolateral prefrontal cortex (BA46) (Selemon et al., 1998) but not in the anterior cingulate cortex (BA24) (Benes et al., 2001) in line with the findings of regional variation of sulcal-specific thinning.

As a marker of supragranular cortical pathology in schizophrenia, sulcal-specific thinning has further implications for understanding the development of schizophrenia. Schizophrenia commonly manifests during adolescence and is thought to relate to structural changes over this period (Insel, 2010). In particular cortical thinning, which is non-uniform in healthy neurodevelopment, demonstrates increased thinning in sulci compared to gyri (Vandekar et al., 2016). Histologically much of layer II merges with layer III of the cortex between late childhood and adulthood (Brodmann, 1909). If healthy adolescent development involves thinning and pruning of dendrites in layers II and III (Woo et al., 1997), for which sulcal thinning is a marker, then excessive sulcal thinning in schizophrenia (White et al., 2003) might be a result of dysregulation of this normal developmental process in adolescents. Indeed sulcal thinning in certain cortical regions has been shown to be a vulnerability indicator in schizophrenia (Goghari et al., 2007).

The fact that these observations suggest widespread supragranular changes is in keeping with the prevailing view that the range of functional changes in schizophrenia is diffuse in origin. The fourth observation - of a steeper thickness gradient in the visual hierarchy in
Multiple markers of cortical morphology reveal evidence of supragranular thinning in schizophrenia - may be interpreted in functional terms, notably with respect to the balance between top down and bottom up processing, which is a key part of the predictive coding model of schizophrenia (Fletcher and Frith, 2009). This model hypothesizes a widespread disruption, whereby the comparison between feedback predictions and feedforward sensory input to create a prediction error is perturbed. Across the cortex this comparison is made in the supragranular layers II/III (Bastos et al., 2012) and multiple lines of evidence suggest that this supragranular function is disrupted in schizophrenia. Prediction errors are communicated at gamma-range oscillations (Bastos et al., 2015; Buffalo et al., 2011; Fontolan et al., 2014), which are widely disrupted in schizophrenia (Williams, 2010). Moreover patients fail to modulate their response from unpredictable to predictable stimuli (Baldeweg and Hirsch, 2015; Fogelson et al., 2011; Urban et al., 2008), suggesting differing feedback signals are failing to modulate prediction error signaling. The final result demonstrates that the normal progressively changing structure of the cortex within a sensory hierarchy is disrupted in schizophrenia. Cortical hierarchies show progressive changes in cytoarchitecture and thickness, reflecting a shifting balance between feedforward and feedback connectivity (Wagstyl et al., 2015). A steepening visual hierarchy suggests a shift towards stronger feedback connectivity – such a shift has also been demonstrated using fMRI (Fogelson et al., 2014). These results therefore offer an important link between the observation of post mortem supragranular layer pathology and widespread functional deficits in prediction error coding in schizophrenia.

In vivo imaging cannot yet resolve these laminar cortical changes directly, but here it was demonstrated that certain markers of such changes can be quantified at the relatively low resolution accessible to 1.5 T or 3 T MRI. Indeed as these are four complementary and cytoarchitecturally derived markers, they are more robust to problems with false positives that beset vertex or voxel-wise analyses. Although their applicability was demonstrated in schizophrenia, the methods developed here may be applied generally. For example cortical layers follow different developmental trajectories (Conel, 1939) that may produce differential development of both cortical regions (Shaw et al., 2008) and of gyri and sulci (Vandekar et al., 2016). As such, the methods presented here may be adopted as useful markers of cortical development. Similarly many other neuropsychiatric cortical pathologies exhibit a degree of laminar specificity, including Alzheimer’s disease (Lewis et al., 1987), and autism (Stoner et al., 2014). The power to identify subtle case-control differences in these diseases may potentially be increased by adopting surrogate markers of laminar specific changes.
3.5 Discussion

3.5.1 Conclusions

Morphological measures in structural MRI are necessarily limited by scale. However by using fundamental knowledge of cortical organization, I developed a series of surrogate markers of supragranular layer changes. Although the value of the methods developed here has been demonstrated in schizophrenia, they are as generally applicable as other, more conventional approaches to studying cortical morphology. These in vivo structural results are consistent with neuropathological, developmental and functional evidence that schizophrenia is characterised by widespread abnormalities in the supragranular cortical layers.

3.5.2 Acknowledgements

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Chapter 4

Mapping cortical laminar structure in the 3D BigBrain

4.1 Abstract

Histological sections offer high spatial resolution to examine laminar architecture of the cerebral cortex. However, it is restricted by its 2D nature hence only regions with sufficiently optimal cutting planes can be analyzed. Conversely, non-invasive neuroimaging approaches are whole-brain but have relatively low resolution. Consequently, 3D cross-cortical patterns of laminar architecture have never been mapped in histological sections. I developed an automated technique to identify and analyze laminar structure within the high-resolution 3D histological BigBrain. White matter and pial surfaces were extracted, from which were derived histologically-verified surfaces at the layer I/II boundary and within layer IV. Layer IV depth was closely correlated with cortical mean curvature (r=-0.76, p<0.0001), but varied by 30% between areas. This fully automated 3D laminar analysis is an important requirement for bridging high-resolution 2D cytoarchitecture and in vivo 3D neuroimaging. It lays the foundation for in-depth, whole-brain analyses of cortical layering.

4.2 Introduction

The isocortex, which forms the major part of the human cerebral cortex, has six layers, where the properties of layers vary between cortical areas (Brodmann, 1909). Individual layers exhibit differing cellular composition and distributions (von Economo and Parker, 1929), developmental trajectories (Conel, 1939), connectivity (Rockland, 2015), physiology (Douglas and Martin, 1991) and functional roles (Bastos et al., 2012). To date, quantitative mea-
Measurement of laminar structure has required manual delineation of the layers on histological sections, which is time-consuming, two-dimensional and largely observer-dependent, with a few exceptions (Schleicher et al., 1999). While whole-brain non-invasive neuroimaging is beginning to resolve laminar-scale features (Bastiani et al., 2016; Fracasso et al., 2016; Yann et al., 2015), the layers identified do not necessarily correspond to cytoarchitectural layers, nor is the resolution sufficient to analyze cellular architecture. Thus, patterns of cytoarchitecture across the entire brain have not hitherto been characterized. I have therefore developed a fully automated method to identify cortical laminar structures within the BigBrain, a 3D high-resolution histological dataset (Amunts et al., 2013), and to comprehensively quantify classically observed patterns of laminar structure. Such a capability opens new vistas in our understanding of laminar patterns across the brain and, moreover, if we can use non-invasive imaging approaches to laminar analyses, it creates the potential for novel insights into structure-function relationships and of characterizing pathophysiology.

Classical histological atlases are the primary source of information on cytoarchitecture, as current non-invasive imaging techniques cannot readily resolve cytoarchitectonic layers. For example, one principle drawn from 2D histological sections was that laminar structure appears to be related to the cortical folds. In particular, the upper cortical layers are thinner at the top of gyri, and thicker in sulci while lower cortical layers show the inverse relation (Bok, 1929; von Economo and Koskinas, 1925). Furthermore, both total and laminar cortical thicknesses vary from area to area, in a way that is systematically related to cytoarchitecture, connectivity and functional specialization (Hilgetag and Grant, 2010; von Economo and Koskinas, 1925; Wagstyl et al., 2015). Drawing on these principles, it has been possible to better localize the laminar origin of structural and functional signals measured in vivo (Kok et al., 2016; Muckli et al., 2015; Waehnert et al., 2016; Wagstyl et al., 2016).

In order to fully exploit histological measurements made in 2D, they must be represented in 3D. However, their application to volumetric in vivo MRI is beset with problems. First, measurement is carried out on 2D sections of a 3D curved object, which introduces limits to measurement and errors due to the angle at which the section intersects the cortex. Second, there are also difficulties associated with registering restricted tissue sections back to the entire cortex. Third, manual measurement is time consuming and highly observer dependent (von Economo and Koskinas, 1925), which places limits on the number and reliability of recorded samples. Together, these manual and two-dimensional limitations have made it difficult, if not impossible, to obtain whole-brain models of the cortical layers. Therefore, while histological studies provide detailed high resolution insights into cortical neuroanatomy, they require extrapolation from a limited number of measurements. There is therefore a pressing need for automated methods that measure 3D laminar structure comprehensively.
The BigBrain is a unique high resolution, comprehensive 3D histological model of a complete human brain, including the cerebral cortex (Amunts et al., 2013)(https://bigbrain.loris.ca/). The original 2D coronal sections were stained for cell bodies before being digitized and reconstructed into a 20µm isotropic 3D volume wherein it is possible to visualize bands of cell bodies corresponding to cortical layers in three orthogonal planes or any oblique angle (Borgeat et al., 2007). I therefore sought to develop a 3D surface-based, automated method for quantitative analysis of laminar cytoarchitecture (Figure 4.1). White and pial cortical surfaces, similar to those used for in vivo MRI imaging, had already been reconstructed for the BigBrain (Lewis et al., 2014). Based on these, profiles of staining intensity were first extracted from pial to white surfaces at all vertices. These were tested to establish the minimum resolution required to resolve layers, and the degree of anisotropic smoothing required to identify laminar-specific changes in staining intensity. Next, two surfaces related to cortical layers were identified - the boundary between layers I and II and a surface within layer IV. The layer I/II boundary was chosen as it commonly used as the upper bound in analyses of cortical cytoarchitecture (Schleicher et al., 1999, 2005), as layer I exhibits little interregional variability. Layer IV was chosen as important division between supragranular and infragranular layers, which have different connectivities and functional roles (Bastos et al., 2012; Felleman and Van Essen, 1991; Markov et al., 2014). Importantly, these layers exhibited relatively consistent features in simulated profiles between cortical regions derived from neuronal density measurements made on 2D sections in the von Economo atlases (von Economo and Koskinas, 1925). Critically these automatically-defined surfaces were then verified through manual histology. Cortical reconstructions were used to measure cortical geometry, specifically curvature and layer thicknesses, to test the relationship between laminar structure and cortical folding across the entire cortex, and mapped the inter-regional variation in the position of cortical layers. Finally, the angles between cortical profiles and the original coronal sections were measured to estimate the measurement errors and limitations for equivalent 2D analyses.
Fig. 4.1 Automatically identified cortical layers on the BigBrain displayed on three orthogonal planes. The brain was sectioned coronally and reconstructed to create a 3D isotropic volume at 20µm. Cortical intensity profiles (zoomed insert), perpendicular to the cortex, were extracted at all vertices on the surface, and used to identify continuous cortical layers.
4.3 Methods

4.3.1 Data preparation

BigBrain is a 20x20x20µm (henceforth described as 20µm) resolution volumetric reconstruction of the histologically processed post mortem brain, which in full is approximately 1TB in size. Running computations on this amount of data can be achieved using a combination of two methods - subsampling to lower resolutions or dividing the data into manageable blocks. Thus, the data was subsampled at a range of isotropic resolutions 40µm, 100µm, 200µm, 300µm, 400µm. Between 100µm and 1000µm the data could be analyzed as a single volume. For 40µm, the data was stored into 125 individual blocks, corresponding to five subdivisions in the x, y and z directions, with overlap. The overlap of blocks was calculated to be sufficient such that a single cortical column could be located in a single block, enabling extraction of complete intensity profiles between pairs of vertices at the edge of blocks without intensity values being altered by boundary effects when the data were smoothed.

4.3.2 Tissue classification

The initial tissue classification was performed on images of the 20µm coronal sections. Voxels were classified into six tissue types using an artificial neural network trained on manually identified example voxels (Zijdenbos et al., 2002). These included cortical layer I, cortical gray matter (layers II-VI), white matter, subcortical and brainstem gray matter, pineal gland, and cerebellar granular layer (Lewis et al., 2014). Layer I was generally separable as it has white matter-like intensities but is disconnected from white matter. The data for the 20µm sections were too large to be reconstructed as a single volume, so the histological and classified sections were assembled into a single volume at 200µm, which was deemed sufficient for the purpose of cortical gray and white surface extraction.

4.3.3 Cortical surface reconstruction and registration

High-resolution polygonal mesh surfaces were fitted to the gray-white matter boundary based on the tissue classification at 200µm. The white surfaces were then locally adjusted to the maximum intensity gradient within the 200µm histological volume. Errors in the white matter surface, due to factors such as technical artifacts (e.g., remaining tears in the sections, and small localized staining artifacts) and tissue misclassifications, were manually corrected on the 40µm blocks. The white surfaces were subsequently expanded to the gray-matter/cerebrospinal fluid (csf) boundary using the CLASP algorithm (Kim et al., 2005). The
resulting white and pial matter surfaces each contain 163,842 vertices per hemisphere, with each pial matter vertex being linked to its homologous vertex on the white matter surface. Morphological landmarks - cortical gyri and sulci - were used to register these surfaces to an average template surface created using in vivo structural MRI from 152 healthy human controls (Evans et al., 2012; Lyttelton et al., 2007). Thus cytoarchitectural information from the BigBrain can be readily mapped to in vivo neuroimaging data.

4.3.4 Cortical staining intensity profiles

Profiles of staining intensity throughout the cortical depth were created by sampling the BigBrain volumes at 100 equidistant points between linked vertices on the pial matter surface and the white matter surface (Schleicher et al., 1999). Given the size of the full resolution dataset, I investigated the minimum voxel size required for cortical layer identification. To this end, sample profiles were extracted for a vertex in the primary visual cortex (V1) in the calcarine sulcus at isotropic resolutions of 20µm, 40µm, 100µm, 200µm, 300µm, 400µm, 1000µm. The staining intensity of volumes with a voxel resolution between 100-1000µm were sampled from a single image volume, whereas for 20µm and 40µm they were sampled for the overlapping blocks.

4.3.5 Volumetric smoothing

Data were anisotropically smoothed, predominantly along the tangential direction, to maximize interlaminar intensity differences while minimizing the effects of intralaminar intensity variations caused by artefacts, blood vessels and individual neuronal arrangement, e.g. in small clusters. The full-width half maximum (FWHM) of the Gaussian smoothing kernel in a given direction was weighted by the inverse of the gradient in voxel intensity in the same direction. Thus across layer boundaries, where the change in intensity is the greatest, there was minimal smoothing, whereas within cortical layers, where there are minor fluctuations due to non-uniform cell distributions and noise, smoothing was maximal (Figure 4.4). The optimal smoothing kernel was chosen as the minimum FWHM where the number of peaks in a set of taken profiles from distributed samples (frontal, occipital, parietal and temporal lobes) was between 3 and 5, approximately the number of peaks expected due to 6-8 cortical layers. These remaining peaks are likely due to interlaminar differences (Figures 4.4 & 4.5).
4.3 Methods

4.3.6 von Economo profiles

The BigBrain dataset is based on histological sections which were silver-stained for neuronal cell bodies (Merker, 1983). The cell bodies are more heavily stained, while the neuropil remains unstained. Thus changes in voxel intensity are primarily related to cellular packing density (Devlin and Poldrack, 2007; Wree et al., 1982). To interpret the cortical profiles obtained in BigBrain, I simulated density profiles based on the manual measurements of neuronal density and thickness of each cortical layer in multiple areas carried out by von Economo and Koskinas (Figure 4.6) (von Economo and Koskinas, 1925). These authors provided numbers for layer thickness and neuronal packing density, which served as a basis to create a histogram-like profile, where the height was given by the density of a layer and the width by the thickness of that layer. For visual comparison, these histograms were then smoothed to the same degree as the smoothed BigBrain intensity profiles. Von Economo density profiles were simulated for 30 areas for which measurements were available. Cortical staining intensity profiles were sampled from manually identified corresponding areas in the BigBrain. While there is uncertainty as to the precise location of areal boundaries relative to morphological features (Amunts et al., 2007b), one can be more confident of the approximate centre of cortical areas. For example, von Economo area FA is located in the postcentral gyrus, while PA is at the base of the central sulcus. Guided by morphological approximations of the locations sampled by von Economo and Koskinas, I manually identified vertices within each of the corresponding areas on the surface of the BigBrain and extracted the cortical intensity profiles for each (Figure 4.6). The von Economo and BigBrain profiles were then visually compared for profile features that were consistent across most cortical areas and which could be used to identify cortical layers automatically on the BigBrain intensity profiles.

4.3.7 Identification of cortical layers

Cortical intensity profiles extracted between white matter and pial surfaces were used to identify cortical layers. Based on the von Economo profile features, the two most common laminar features were a sharp rise in neuronal density at the boundary between layers I and II, and a peak in neuronal density at the centre of layer IV (Figure 4.6).

The layer I/II boundary was identified as follows: profiles were created sampling the tissue classification (200µm isotropic voxels) and histological staining intensity (40µm anisotropically smoothed) at 100 points between the pial and white surfaces. The first estimate was placed at the innermost layer I-classified voxel or, when no appropriately classified voxel was found, 200µm below the pial surface (Figure 4.2). This was then
adjusted to the position of the closest maximum gradient of the staining intensity profile. The intensity at this maximum gradient position was then calculated at each vertex and smoothed by three iterations of nearest-neighbour averaging, to remove noise from isolated misplaced vertices. The surface was then adjusted to the point in the profile that matched this smoothed intensity value. When no matching intensity value was found or its position was deeper than the expected thickness of layer I (200µm), vertices were adjusted to the average depth of their neighbours (Figure 4.2) and the search for the position of the closest maximum gradient was repeated.

Bounded by the layer I/II boundary surface and the white matter surface, layer IV was initially identified as a large peak in intensity, which followed an inflection point corresponding to part of layer II/III (layer II is often but not always a peak, thus an upward inflection point more reliably marks layer II/III in the profile). To account for noise artefact and staining inhomogeneities, the surface mesh was smoothed iteratively in two ways. The first was geometric smoothing of the mesh without shrinkage (Taubin, 1995). The second was by calculating equivolumetric depth of this interim layer IV surface between the layer II and white matter surfaces (Figure 4.2)(https://github.com/neurospin/highres-cortex) (Bok, 1929; Waehnert et al., 2014; Yann et al., 2015). Equivolumetric cortical depth was based on the 200µm tissue classification volume, and cortical voxels were given intensity values which represent the fractional equivolumetric depth between the white and layer I/II surfaces. Thus sampling the intensities at the intersection with the interim layer IV surface gives the fractional depth free from morphological or mesh distortion that affect Euclidean distance measurements. These depth values were then smoothed across the surface with a Gaussian kernel of 10mm FWHM (Boucher et al., 2009). The resultant, smoothed estimates for layer IV depth were then used as an estimate of layer IV position within the cortical profile. This estimated depth was used as a starting point to find the nearest peak in intensity for each vertex to generate a new layer IV surface. The 3 steps were repeated until fewer than 100 of the 163842 cortical vertices changed location between successive iterations.

### 4.3.8 Validation through manual delineation of histology sections

To test the accuracy of layer I/II and layer IV surface placement, automatically identified cortical layers were compared against manually delineated cortical layers carried out on a subset of the original 2D histological sections of the BigBrain rescanned at 5µm resolution, which allows clear identification of single cells. Samples were identified on 7 sections, chosen from those available at 5µm, to have several suitable portions of cortex in different cortical areas. Sections 1066, 2807, 3300, 3863, 4366, 4892, 5431 from the total of 7404 were included, representing a range of positions from caudal occipital to rostral frontal. On each
Fig. 4.2 a. Automatically detected layers from cortical profiles between pial and white surfaces. b. An initial estimate for layer I/II boundary position was placed at the layer I/gray boundary of the tissue classification, made on 200µm isotropic voxels (yellow/gray voxels). Surfaces were adjusted to the nearest maximum gradient, with two iterative smoothing steps to produce a smooth surface following the visible boundary between these layers: intensity values were smoothed across the surface and vertices were moved to the position on the profile nearest the smoothed value, and geometric mesh smoothing removed high curvature kinks by averaging neighbouring coordinates - as shown by the green dotted line. c. The initial position of layer IV was placed at the first major peak after an upwards inflection in the intensity profile, which corresponds to layer II/III. Surfaces were adjusted to the nearest profile maximum, with two iterative smoothing steps: equivolumetric depth potential values (spectral-coloured 200µm voxels) were smoothed across the surface to reposition smooth but inaccurately placed regions (purple dotted line) and geometric mesh smoothing was again used to remove high curvature kinks.
of the 7 sections, 6 sample regions were chosen where a number of cortical intensity profiles were within 5 degrees from the plane of the section, thereby minimizing errors due to oblique cutting planes. For each sample structures (the border between the white matter and cortex, pial, layer I/II boundary and the upper and lower limits of layer IV) were manually delineated. In six of the 42 sample regions, the borders of layer IV were not clearly visible. For two of these, layer IV was reduced ("dysgranular") to such a degree that a single line was used to label boundary of layers III and V. This was not possible in the final four regions ("agranular"), in which layer IV was not visible, and These samples were therefore excluded. A further sample was omitted due to excessive tissue tearing in the original section. The seven sections containing the remaining 37 manually delineated regions were then registered to the aligned 3D BigBrain volume for comparison with the automatically identified layers. In addition to visually comparing the layers, two statistical tests were carried out to verify cortical layer placement. First, absolute distance was calculated between the manually delineated layers and the coordinates at which the two automatically identified surfaces intersected the plane. After testing the distance error for normality with the Anderson-Darling test, a t-test was carried out on the mean distance for each sample to test whether the error differed from zero - i.e. whether a different surface was being systematically identified. Second I tested whether the local variations in layer IV position, which were strongly related to cortical curvature (Figure 4.9), matched the variations of layer IV position in the manual annotations. For the manual segmentations, relative depth of layer IV was calculated by measuring the distance from a line mid-way between the upper and lower limits of layer IV to the layer I/II layer and white matter surface. Pearson’s correlation coefficient was calculated between relative depth of the automated layer IV and the manually delineated layer IV, for each sample.

4.3.9 Morphological and inter-regional variations

The relative depth of layer IV at each vertex was correlated against mean cortical curvature to quantify in 3D curvature-dependent changes in the position of layer IV. This relationship was first hypothesized in classical studies of 2D sections (Bok, 1929; von Economo and Koskinas, 1925). Mean cortical curvature was calculated at each vertex on a mid-surface which was equidistant between white and layer I/II surfaces. Similarly the percentage depth of layer IV, taken between the layer I/II and white matter surface, was calculated for each vertex. Both sets of data were smoothed with 3mm FWHM Gaussian kernel, to remove isolated extreme values. Surfaces were then masked to exclude medial wall and hippocampal vertices, and vertices where the cortical thickness seemed to be smaller than a biologically implausible 0.5mm - these values generally occurred where the cortex was damaged due to histological processing. Pearson’s correlation coefficient was calculated between curvature and layer IV
depth across all cortical vertices. Finally, a linear model was used to regress out the effect of mean curvature on depth, to examine residual cross-cortical variability in layer IV depth.

### 4.3.10 Analysis of cortical shape

The 3D coordinates of paired pial and white matter vertices were used to calculate the angle at which they intersect the coronal sections and the amount of error this would introduce if measured with 2D cortical histology. The angle $\alpha$ is given by:

$$\arcsin \left( \frac{N_{\text{section}} \cdot (v_{\text{pial}} - v_{\text{white}})}{v_{\text{pial}} + v_{\text{white}}} \right)$$

(4.1)

where $N$ is the normal vector to the section plane and $v_{\text{white}}$ and $v_{\text{pial}}$ are the coordinates describing pial and white vertex locations. The overestimation error introduced when measuring the thickness of a piece of cortex where the profile intersects the plane at an angle $\alpha$ is given by:

$$\left( \frac{1}{\cos(\alpha)} - 1 \right) \cdot 100$$

(4.2)

### 4.4 Results

#### 4.4.1 Voxel resolution and smoothing parameters:

Increasing voxel resolution revealed progressively clearer layer-related variation in cortical intensity (Figure 4.3). Layer-related intensity peaks were first visible at around 400\(\mu\text{m}\) and became increasingly well-defined as the resolution was increased to 20\(\mu\text{m}\). However noise related fluctuations in intensity also increased, such that at 20\(\mu\text{m}\) there were dozens of peaks in intensity, many within the same cortical layer. Layer-related changes in cortical intensity were sufficient at 40\(\mu\text{m}\) to identify major laminar features, which provided an 8-fold reduction in data volume enabling greater computational efficiency relative to 20\(\mu\text{m}\) volume. The same level of laminar detail was not visible at the lower resolutions of conventional in vivo MRI. To minimize the level of noise, data were anisotropically smoothed with the smoothing kernel weighted by the inverse of the intensity gradient, with maximal smoothing in the tangential direction and minimal smoothing across layers in the radial direction (Figure 4.5). A range of anisotropic smoothing kernels was tested against number of cortical profiles sampled from across the cortex (Figure 4.4). The degree of anisotropic smoothing is determined by the number of iterations, with the effective FWHM being calculated post-hoc. At 0.163mm maximum FWHM smoothing, the mean number of peaks was between 3 and 5.
This represented an optimal balance between differentiating laminar peaks and minimizing noise-related intensity changes. Subsequent analyses were therefore carried out on 40µm isotropic voxels, anisotropically smoothed at a maximum FWHM of 0.163mm (Figure 4.5).

Fig. 4.3 Intensity profiles at subsampled resolutions between 1000µm and 20µm from pial to white vertices within V1. 20µm and 40µm provide sufficient resolution to resolve laminar features, with the 8-fold reduction in data volume at 40µm enabling greater computational efficiency. Layer-related intensity peaks are still evident around 300µm, close to the current limit for in vivo MRI. Profiles generated at 40µm resolution, anisotropically smoothed at 0.163mm maximum FWHM exhibited sufficient intralaminar smoothness and interlaminar contrast for subsequent layer identification. Profiles were translated on the y-axis for visualization purposes.

### 4.4.2 Characteristic laminar features of cortical profiles

Comparison of profiles from neuronal density changes in von Economo neuronal density (number of cells per area) and BigBrain intensity profiles revealed characteristic changes in intensity. The most characteristic, consistent feature was a sharp change in intensity at the boundary between layers I and II. This was found in all von Economo profiles and was clearly visible in histological sections. The corresponding feature on the BigBrain profiles was a peak in the first derivative of the profile, close to the pial surface. The second most characteristic feature was a large peak in neuronal density at the centre of layer IV, the granular layer. While this peak was not present in all samples, particularly in the "agranular" cortices (e.g. area FA, the primary motor and premotor areas (Zilles et al., 2015a)), it was present across most other cortical areas (Figure 4.6). The corresponding intensity peak was
Fig. 4.4 Anisotropic volumetric smoothing kernel was varied from 0-0.25 mm maximal FWHM on blocks of 40µm resolution. Profiles in frontal, parietal, temporal and occipital lobes were sampled at each of these degrees of smoothing. Without smoothing, profiles had many additional peaks due to finer architecture. The kernel of 0.163mm was chosen as it was the minimal kernel where profiles had between three and five peaks, which was the approximate range expected given the six layers of the isocortex. Moreover these peaks appeared stable when the FWHM was increased, further reducing the likelihood that they were in fact noise artefacts.
Fig. 4.5 Comparison of unsmoothed, isotropically smoothed and anisotropically 40µm resolution data in the calcarine sulcus (V1). The unsmoothed profile had many minor peaks and troughs obscuring the major laminar features. The profile taken from the data smoothed isotropically with a Gaussian kernel of 0.163mm FWHM showed peaks corresponding to the 8 expected layers (I, II, III, IVa-c, V, VI), but with reduced intralaminar contrast, whereas the profile sampled from anisotropically smoothed data (maximum FWHM 0.163mm) showed the same peaks but with greater contrast.
then identified in the BigBrain intensity profiles. Comparison of BigBrain staining intensity profiles with von Economo histological numerical density profiles revealed features that were consistent across most cortical areas and which could therefore be used for automated identification of cortical layers from intensity profiles (Figure 4.6).

Fig. 4.6 a. Cellular density profiles were created from areal measurements of thickness and neuronal density of each cortical layer reported in von Economo & Parker, 1929 (von Economo and Parker, 1929). The first letter of each areal code corresponds to the lobe, the second letter indicates successive lobar measurements e.g. OA - occipital area A, TB - temporal area B etc. All von Economo profiles exhibited a large positive gradient between layer I, which has the lowest neuronal density, and layer II. Most profiles also exhibited a consistent mid-profile peak corresponding to high neuronal density in layer IV. A notable exception included FA, the agranular motor cortex, so called because no granular layer is visible under a microscope. b. von Economo density profiles were compared with intensity profiles from corresponding areas in the BigBrain. Shown here are von Economo areas OA (extrastriatal occipital cortex) and FD (granular anterior frontal cortex). The layer I/II boundary and mid-cortical layer IV peak is consistently present in both von Economo and BigBrain profiles.

4.4.3 Verification of automatically identified cortical layers

Across the 37 samples, automatically identified layer I/II boundary and layer IV (Figure 4.2) closely followed the manually delineated layer bounds (Figure 4.7). The absolute distance from the manual delineations was calculated for each vertex within a sample region of interest. These error distances were averaged across the sample to give a mean error for each of the
37 regions. Signed mean distance was calculated to assess for systematic biases in the layers being identified, while unsigned mean distance was calculated to assess the consistency of the accuracy. For signed mean distance, negative distance values indicated the automated surface was closer to the white matter surface than the manually delineated boundary and positive distance values indicated the surface was closer to the pial surface.

The mean distance between manually and automatically identified layer I/II boundary across all samples was -13\(\mu m\) (s.d. 40\(\mu m\), range -120\(\mu m\) to 70\(\mu m\), unsigned error was 63\(\mu m\)). In order to put this distance in context, these surfaces were calculated on 40\(\mu m\) resolution data. The Anderson-Darling test indicated that the data were normally distributed (\(A=0.45\), \(p=0.26\)) and a one sided t-test indicated that the mean error did not differ significantly from zero (\(t=-1.78\), \(p=0.08\)). Thus there was no evidence of a systematic error in the identification of layer I/II. For layer IV, the mean error across all samples was -11\(\mu m\) (s.d. 68\(\mu m\), range -161\(\mu m\) to +140\(\mu m\), unsigned error was 72\(\mu m\)) (Figure 4.7). The Anderson-Darling test indicated that the data were normally distributed (\(A=0.23\), \(p=0.80\)) and a one sided t-test indicated that the mean error did not differ significantly from zero (\(t=-1.03\), \(p=0.31\)). Again, there was no evidence of a systematic error - the layer being identified was within layer IV. For each sample, the percentage depth of layer IV between layer I/II boundary and the gray/white boundary correlated strongly with the percentage depth of the middle of the manually delineated layer IV (across all samples mean Pearson’s \(r = 0.72\)) (Figure 4.7). Per-sample measurements of regional accuracy mapped to the cortical surface can be found in (Figure 4.8). Therefore, the automatically defined layer IV closely followed the within-area and between area variations in position of the manually delineated layer IV.

### 4.4.4 Layer IV depth and cortical morphology

The relationship between cortical morphology and cortical laminar structure was quantified, a relationship which was first noted in 2D histological studies (Bok, 1929; von Economo and Koskinas, 1925) (Figure 4.9). Testing across all cortical vertices on both hemispheres, the depth of layer IV was highly correlated with the mean curvature of the cortex, as measured on a mid-surface between the layer I/II boundary and white surfaces (Pearson’s \(r =-0.76\), \(p<0.0001\)) (Figure 4.9). In other words, layer IV was more superficial in gyri and deeper in sulci. This finding was consistent with classical histological studies including the equivolumetric model of laminar structure, and provided 3D evidence that upper cortical layers (I-III) are thinner at the top of gyri, but thicker in sulci whilst lower layers (V-VI) show the inverse relation. This relationship was present across the entire cortex, and therefore must be considered when sampling cortical tissue either with histological techniques or for in vivo MRI.
4.4 Results

Fig. 4.7 a. Distance of layer IV vertices from layer IV boundaries manually delineated in 37 areas on 5 μm histological sections. Most points lie directly within or close to layer IV and there is no apparent systematic bias in the small number of points lying outside of these bounds. b. Verification that local, morphologically determined variability in automatically identified layer IV depth followed that of the manually delineated layer IV bounds, shown here for six samples on a single coronal section: 2807. The percentage depth of the midpoint of the manually delineated layers between the white and layer I/II boundary and the percentage depth of the automatically identified layer IV between automated white and layer I/II surfaces was compared for each sample region. The mean Pearson’s correlation coefficient for this section was r=0.80 (across all sections r=0.72). c. Visual validation that layer I/II and layer IV closely follow manually defined boundaries for section 2807 at 20 μm. The automatically identified surfaces have the following colours: white - red, layer IV - burgundy, layer I/II - green, pial - blue. The white-blue overlays indicate distance below the manually defined layer IV/V boundary, with the lower bound at the white surface. The white-red overlays indicate distance above layer III/IV boundary, with the upper bound at the layer I/II boundary. The automatically identified layer IV surface consistently lies within or close to layer IV.
Fig. 4.8 Mean distance between automatically identified layer IV and manually delineated layer IV bounds for 37 sample regions, mapped to cortical surfaces which have been heavily smoothed to enable viewing of deep sulcal fundi. Generally, the mean error was within 100 µm, except for agranular primary motor cortex and cingulate cortices, for which layer IV is particularly abnormal or even absent, and anterior temporal lobe, which on subsequent inspection was likely caused by cuts to the cortex made by the saw in the original brain extraction. As validation was carried out on single sections, surface mappings of samples were expanded for visualization purposes. Nevertheless, not visible on these images were two buried right insular regions, for which the mean errors were 62 µm and 34 µm.
Fig. 4.9 a. Relative depth of automatically identified layer IV displayed on a spatially smoothed white surface. Consistent with histological studies (Bok, 1929), layer IV was located deeper in sulci and more superficial in gyri. Upper layers (II & III) were therefore relatively thinner in gyri, and thicker in sulci, while the opposite was true for lower cortical layers (V-VI). b. Layer IV percentage depth was strongly correlated with mean curvature measured on a mid-surface between layer I/II and white surfaces (Pearson’s r=0.76, p<0.0001), being deeper in negatively curved sulci and more superficial in positively curved gyri.
4.4.5 Residual cross-cortical variability in layer IV depth

The relationship with mean curvature was linearly regressed from the individual vertex layer IV relative depths to assess whether there was residual layer IV variability across cortical areas. After linearly regressing out the effect of mean curvature on layer IV depth, there remained considerable residual variability position of layer IV depth (Figure 4.10) - accounting for curvature, layer IV depth varied up to 30% between areas. Thus the relative depth of layer IV is determined both by local morphology and inter-regional differences in cytoarchitecture.

![Residual layer IV relative depth](image)

Fig. 4.10 Residual layer IV relative depth after regressing for the relationship with curvature, overlaid on the pial surface. Across cortical areas there remained a variability in layer IV relative depth of around 30%. Layer IV was relatively deeper in the calcarine sulcus (V1) and parts of the medial and dorsolateral prefrontal cortices.
4.4.6 3D histology

Analysis of the angles at which cortical profiles intersected the coronal sections demonstrated how the complex shape of the cortex affects histological measurement (Figure 4.11). Angles ranged from 0°, where no systematic overestimation of thickness if found, because the section is parallel to the cortical profile, to 90°, where the profile is exactly perpendicular to the plane. In the latter case, no thickness measurement would be possible. In-between these extreme values, oblique profiles would have been measurable, but with a systematic error. For example, 69% of vertex profiles had angles above 18°, where the thickness measurement would be 5% overestimates of in-plane measurements of same piece of cortex. Importantly, for most 2D histology these measurements cannot be readily adjusted to account for this error, as computational reconstruction of the cortical surfaces was required to estimate these angles.
Fig. 4.11 a. Estimated 2D thickness measurement error of a 3mm cortex due to the angle between the coronal plane and cortical profiles. Thickness measurements made at vertices coloured blue and purple would be little affected by oblique slicing. Frontal and occipital poles showed many profiles approaching 90° such that a single cortical column would be distributed across hundreds of coronal sections making laminar analysis impossible in 2D. b. Histogram showing the distribution of angles across vertices. At angles above 18°, which made up approximately 69% of vertices, there was a 5% overestimation in any 2D thickness measurement. c. Calculation of the error in thickness measurement associated with a given profile-section angle.
4.5 Discussion

I have developed and validated a fully-automated 3D analysis of the laminar structure of the cerebral cortex in the BigBrain. This enabled the identification of two layers across the entire cerebral cortex and revealed cross-cortical patterns of cytoarchitecture. These results demonstrated that features of cortical staining intensity profiles corresponded to specific cortical layers and, at 40µm isotropic resolution, these can be used to identify surfaces automatically at the layer I/II boundary and within layer IV (Figure 4.1). The accuracy of layer extraction was subsequently corroborated through manual histology. Based on these layers, I have quantified the systematic relationship between cortical layer IV depth and cortical mean curvature, and the extent to which the relative depth of this cortical layer changes among cortical areas. These findings have implications for our understanding of cytoarchitectural organization and future directions for in vivo cortical neuroimaging. In particular, they offer exciting possibilities for more precise characterizations of the nature of structural changes associated with neuropsychiatric conditions and for linking such characterizations to functional disturbances.

The voxel resolution of human MRI currently ranges from 1mm (1000µm) in vivo to around 200µm post mortem. BigBrain is a 20µm isotropic volumetric dataset. Subsampling the data enabled testing of the minimum resolution required to identify lamina-related intensity peaks. The results showed that between 20-40µm, changes in intensity corresponded to specific laminae (Figure 4.3). From 100µm to around 300µm, some peaks still corresponded to those seen at higher resolutions, but below this resolution intensity profiles lose detail. Thus, with an MRI resolution above around 300µm, combined with a sequence where voxel intensity is sensitive to differences in layer composition (Fracasso et al., 2016; Waehnert et al., 2016), this methodology provides a framework to identify the position of certain laminar features which correspond to specific cortical layers in vivo at the individual level. Moreover, this approach has enabled quantification of the systematic inter-regional and morphological changes in layer depth across the cortical surface.

40µm anisotropically smoothed intensity profiles were used to identify comprehensive cortical layers; at the boundary between layers I and II and a mid-cortical peak in intensity which corresponded to layer IV. The position of the automatically-identified layer IV closely varied with the curvature of the cortex (Figure 4.1 & 4.9), such that at the top of gyri it was closer to the pial surface and at the bottom of sulci it was closer to the white matter surface, this finding being consistent with known cortical anatomy (Bok, 1959; von Economo and Koskinas, 1925). These findings further validate the equivolumetric model of laminar structure, which has been used to predict the positions of cortical layers, in in vivo MRI, from the curvatures of the white and pial surfaces (Bok, 1936; Waehnert et al., 2014). Layer IV
Mapping cortical laminar structure in the 3D BigBrain depth, as with many other aspects of cytoarchitecture (Welker, 1990), varies as much between gyri and sulci within the same cytoarchitectonic area as across cortical areas. Although the precise functional impacts of these differences are unclear, morphology is likely to affect the neurophysiology, connectivity and therefore perhaps even the functional role of the cortex (Hilgetag and Barbas, 2005; Welker, 1990). Furthermore, when modelling cortical layers in vivo, it is crucial to account for morphological position in order to ensure that quantitative sampling is carried out within a single target layer.

After accounting for the close relationship between layer IV position and gyral/sulcal morphology (Figure 4.9), there was remaining inter-regional variability in the depths of cortical layers (Figure 4.10). Inter-regional differences in the thicknesses of upper and lower cortical layers, as well as being an important consideration for in vivo modelling, support the hypothesis that the cytoarchitecture of different cortical areas is associated with its connectivity (Hilgetag and Grant, 2010). Variability in layer thickness can arise from a number of linked microstructural properties, including neuronal density (von Economo and Koskinas, 1925), dendritic arborization (Elston and Rosa, 1998), myelination (Hopf, 1968; Nieuwenhuys et al., 2015) and axonal connectivity (van den Heuvel et al., 2015). For example, within the cortical hierarchies, the infragranular layers are thought to be the origin of cortico-cortical feedback connections, which are relatively sparse in primary sensory regions (Felleman and Van Essen, 1991). In the BigBrain, layer IV was very deep (i.e. close to the white surface) in primary visual cortex, which is already thin (Brodmann, 1909; Wagstyl et al., 2015). This suggests that layers V and VI are particularly thin in the visual cortex (von Economo and Koskinas, 1925), reflecting perhaps the sparsity of cortical feedback connections originating from this area. Further investigation of quantitative cytoarchitecture might help disentangle relationships between laminar thicknesses, intracortical circuitry and inter-areal connectivity.

Analysis of cortical profiles between vertices on 3D surfaces enables cytoarchitectural analyses, such as border detection (Schleicher et al., 2005) and quantitative cytoarchitecture (von Economo and Koskinas, 1925) across the entire cortex, with several advantages over classical 2D histological techniques. Cortical analyses were carried out on over 325,000 vertices; manual measurement of this many samples is simply not feasible. My own manual delineation of 37 sample areas from 7 out of 7404 coronal sections, rescanned at 5µm, required at least 20 hours of work, while automated identification of cortical layers for over 325,000 vertices for the entire cortex was carried out in 40 minutes on a single processor. Moreover, manual measurement is highly observer dependent - for example von Economo estimated an interobserver measurement error of up to 500µm for the placement of the white matter surface (von Economo and Koskinas, 1925). Validation of the automated
surfaces revealed that the mean error in automated layer IV position was 11 µm (Figure 4.7), where reduced accuracy primarily in regions lacking a clear layer IV (Figure 4.8). Surfaces were identified using 40 µm resolution BigBrain data and validated against histological measurements that required 5 µm resolution. Despite this lower resolution, the mean distance between manual and automated was less than the width of one 40 µm voxel. While this is not yet perfect, it offers several advantages over classical histology in being rapid, observer independent and reproducible. These attributes are of great value in exploring larger scale datasets, especially developmental and clinical samples, where structural changes can be subtle.

A major issue in traditional 2D histology concerns the orientation of the plane of section. Once sectioned, 2D histological measurement is restricted to cortical samples cut approximately perpendicularly to the surface and is subject to errors introduced by the angle between the section and the surface. For example, within the BigBrain, 69% of vertices on the cortical surface generated profiles intersecting the coronal plane at 18° or more, which would be associated with a 5% or greater overestimate in an in-plane histological thickness measurement - corresponding to 0.15 mm error on a 3 mm cortex. Furthermore 14% of vertices exhibited an angle of greater than 60°, the angle above which the cortex is considered too oblique for histological analysis (Schleicher et al., 1999). Therefore even if it were feasible to manually and reliably annotate 7404 coronal sections, a large part the cortex would not have been accurately measured using individual 2D sections (Figure 4.11).

Finally, these measurements were carried out on a reconstructed cortical surface, complete with the characteristic pattern of gyri and sulci. This has enabled registration of the surfaces and their associated cytoarchitectural information to an in vivo average template surface (Lyttelton et al., 2007). Thus, patterns of cortical structure gleaned from these data are transferable to in vivo MRI-based cortical reconstructions to generate and directly test novel hypotheses. For example characterizing gyral-sulcal differences in cytoarchitecture can aid interpretation of morphological change (Vandekar et al., 2016; Wagstyl et al., 2016) and accurate sampling of high-resolution BOLD fMRI signal in specific cortical layers has revealed differential functional responses (Muckli et al., 2015).

One limitation from the analysis of post mortem brains is the effect of tissue shrinkage on measurements. While it is possible to account for this artefact (Amunts et al., 2007a, 2005), I was primarily interested in the percentage depth of layer IV, on which shrinkage would have little effect (Amunts et al., 1995). Furthermore there is only a single BigBrain, thus relationships identified here are not necessarily representative of all human brains. Nevertheless morphological and inter-regional differences match and extend those reported...
in classical histological studies, and these insights will aid the future identification of cortical layers in vivo.

4.5.1 Conclusions

I used a fully-automated technique to identify cortical layers in 3D on the BigBrain. There was a close relationship between layer IV depth and morphology across the entirety of the cortex, but layer IV depth varied between regions above and beyond this relationship. These patterns can now be used alongside inter-regional differences to generate strong prior expectations of the location of cortical layers, strengthening our ability to interpret subtle changes in MRI voxel intensity associated with interlaminar differences. Moreover these analysis characterizing the effects of varying voxel resolution of the BigBrain indicated that high-field MRI is approaching resolutions at which, with suitable sequences, identification of certain cortical layers is feasible. Thus the principles of laminar structure derived here, alongside the tools for cortical intensity profile analysis can be readily used to improve translation between cytoarchitectural studies and in vivo cortical analyses.

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Chapter 5

Automated segmentation of cortical layers using convolutional neural networks

5.1 Abstract

The cerebral isocortex has six layered structure that varies depending on cortical area and local morphology. BigBrain is a 3D histological dataset from which valuable insights can be gained through segmenting these layers across the entire cortex. However segmenting these layers is carried out according to histological definitions that are 2D, require expert training and involve time consuming manual delineations. I therefore sought to automate the segmentation of cortical layers in 3D through the application of 1D convolutional neural networks to profiles of intensity extracted through the cortex of the BigBrain. The resultant layers and profile-based segmentation tools have wide-ranging applications through histology to in vivo neuroimaging.

5.2 Introduction

Segmentation of cortical layers is vital for localising and understanding structural and functional differences (Muckli et al., 2015), such as those seen in cortical development (Conel, 1951) and neuropsychiatric disease (Wagstyl et al., 2016), as well as enabling cognitive neuroscience to bridge the micro- and macro-scales. However current in vivo approaches are limited on two fronts - the first is the limited resolution of MRI, the second is not knowing the structure of layers. BigBrain offers a unique dataset to resolve histological
cortical layers in 3D, thereby providing a concrete link between microscale patterns of structure and in vivo markers. This can enable the creation of a whole-brain ground-truth model of cortical layers for neuroimaging approaches (for more information see Introduction and Chapter 4).

Previous work (Chapter 4) has demonstrated that certain consistent image features, seen at 20µm can be used to identify specific cortical layers as identified by histologists at 5µm. However, cortical laminar structure exhibits a large degree of interareal variability (Figure 4.10), such that identifying six cortical layers across the entire cortex requires an algorithm capable of flexibly learning that the same layer can be characterised by different features depending on the cortical area. Furthermore, while certain interlaminar boundaries correspond to features - such as peaks, troughs or sharp gradients - that can be manually identified, not all boundaries have such simple features. As such, convolutional neural networks, which have been used extensively elsewhere in the field of deep learning for feature detection, image classification and image segmentation, might be ideally placed for the problem of cortical layer segmentation.

Neural networks are computational systems modelled on biological networks of neurons (LeCun et al., 2015). Briefly, they are formed of layers of artificial neurons, where each neuron or node in a layer receives input information from a previous layer, applies a non-linear function on a weighted combination of inputs and passes the output of the function on to neurons in the next layer. For supervised learning, a network calculates a predicted classification as the final output from a given a training example. The prediction is then compared with the expertly-labelled target classification and a loss is calculated using a chosen cost function. The weights of the nodes are then updated in a learning step according to the negative gradient of the cost function, and the training step is repeated. This basic network structure has been very successful at many types of classification tasks. For example, in a collaboration with researchers at Great Ormond Street Institute of Child Health, we trained a neural network to detect focal cortical dysplasias in paediatric epilepsy based on cortical morphometric features measured using MRI (Adler et al., 2017). However, to make learning more efficient for difficult tasks, this basic structure can be augmented with various additions that enable the networks to perform certain types of tasks more successfully. In a convolutional neural network, convolutional layers are added in before regular layers, and these have slightly different properties. Within the convolutional layer are multiple filters, each of which are applied across the entire input image space. The filters have a receptive field which is generally larger than one pixel and the weights are altered during the learning step. As each filter is applied across the entire image, they are essentially image feature detectors, searching for specific features across the entire image. In a 1D intensity profile,
learned features might include basic peaks and troughs of intensity but can also become more subtle and complex, far exceeding the possibilities of manual “hand-crafted” features.

However several challenges preclude the simple application of existing convolutional neural network structures to this task. The first is that, in order to learn, neural networks require large amounts of expertly labelled training data. For the 3D BigBrain, the 20µm resolution is insufficient for histologists to identify all cortical layers, but higher resolution images, required for manual segmentation of layers are only available in 2D. Moreover it is simply unfeasible to annotate thousands of 2D histological sections. Therefore I required a method that could create multiple training examples from limited annotated data, and that could interpret equivalently training data created from a 2D image and test data created from 3D volume. These issues were addressed by using 1D cortical intensity profiles. 2D sections were manually annotated and registered to the BigBrain, from which were sampled thousands of labelled profiles from the manually labelled areas. In this way less than 0.0001% of the total volume was annotated, but hundreds of thousands of training examples were created. A 1D convolutional neural network was then trained to identify layers on a 1D intensity profile drawn from the 2D sections, but tested on 1D intensity profiles taken at oblique angles through the BigBrain. This enabled segmentation of cortical layers regardless of obliquity of the original cut with the cortex.
Fig. 5.1 Six cortical layers segmented on the 3D volume on three orthogonal planes. Top right shows the original coronal plane of section. In the bottom left quarter of this coronal plane is an area of cortex where layers would be difficult to segment in 2D due to the oblique sectioning of the cortex.
5.3 Methods

5.3.1 Data preparation

BigBrain is a 20x20x20µm (henceforth described as 20µm) resolution volumetric reconstruction of a histologically processed post mortem human brain (male, aged 65), (available for download at https://bigbrain.loris.ca). In order to run computations on this amount of data, the BigBrain was stored into 125 individual blocks, corresponding to five subdivisions in the x, y and z directions, with overlap. The overlap of blocks was calculated to be sufficient such that each single cortical column could be located in a single block, enabling extraction of complete intensity profiles between pairs of vertices at the edge of blocks without intensity values being altered by boundary effects when the data were smoothed. Blocks were smoothed anisotropically, predominantly along the direction tangential to the cortical surface, to maximize interlaminar intensity differences while minimizing the effects of intralaminar intensity variations caused by artefacts, blood vessels and individual neuronal arrangement (see Chapter 4). The degree of anisotropic smoothing is determined by repeatedly applying the diffusive smoothing algorithm. The optimal level of smoothing was determined in Chapter 4, and gave an effective maximum FWHM of 0.163mm (Figure 4.4). For subsequent analysis both the raw 20µm and anisotropically smoothed blocks were used.

5.3.2 Profiles

Gray and white surfaces were taken as starting surfaces from Chapter 4. These surfaces were extracted at a coarser resolution of 200µm, and were based on image intensity features such as maximum intensity gradient for the white matter surface (Figure 5.2). In contrast, the histological definition of the white matter surface is the point at which cortical neurons are no longer seen. Therefore I expanded the gray and white surfaces used to generate intensity profiles which began outside the cortex and ended well inside the white matter in the following way. Profiles are generally extracted between corresponding vertices on gray and white surfaces, and are thought to approximate the orientation of cortical columns. Correspondence between gray and white surfaces was previously determined by expanding the white surface along the laplace field. This can lead to profiles that are oblique relative to the surface normal and appear to be unconvincing estimates of columnar orientation, particularly in sulcal fundi (Figure 5.2). To address this, an average mid-surface was computed, placing vertices halfway between gray and white surfaces. From each vertex, the nearest point on the gray and white surfaces was calculated. Next the normal vector connecting these nearest points was calculated and the vector components were smoothed...
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across the mid-surface with a FWHM of 3mm. The smoothed “normal” vectors were then used for the generation of profiles, extending the minimum distance in either direction, plus an extra 0.5mm to ensure all the outer surface was outside the pial boundary and the inner surface was deep to the white boundary. The resultant profiles were less oblique and more likely line up with the cortical columns (Figure 5.2).

In order to prevent profiles intersecting in adjacent gyri/sulci a combined chamfer-laplace field was calculated. Within the cortical boundaries, values ranged from 0-10, according to the laplace heat diffusion equation. Outside the surfaces, the field was a scaled chamfer map - voxel values were initially calculated as the mm distance from the nearest cortical surface and then scaled by a factor of three to ensure relatively smooth transitions at the borders with the laplace field. The cerebral cortex is around 2-4 mm thick and the laplace field within this is from 0-10 so 1 mm is roughly 3 units in this field. Inside the white matter, distance values were negative (0,-1,-2 with increasing distance into the white matter), outside the gray matter, they were positively increasing (11,12,13 etc). Inner and outer vertices were then moved along the profiles beyond any extrema in these fields so that profiles from opposing banks of sulci would not overlap causing intersecting surfaces (Figure 5.2).

Extended intensity profiles that are more likely to include histologically defined pial and white boundaries were then created for each pair of vertices from both raw and anisotropically smoothed 20µm volumes.

5.3.3 Training data

Manual segmentations of 6 cortical layers were created on 45 regions of the cortex, distributed across eight original histological BigBrain sections at 5µm. This resolution is sufficient to distinguish cell bodies used to locate specific layers (Figure 5.3a).

Manual segmentations were then co-registered to the full aligned BigBrain space. The manually drawn layers were used to creating corresponding inner and outer surfaces and expanded inwards and outwards between 0.4 and 0.6mm so as to match the test dataset. Training profiles were created sampling raw, smoothed and labelled 20µm data at thousands of profiles per sample. Each layer in the labelled data had a class value (1-6) and the background was set to 0 (Figure 5.3b). This greatly expanded the training dataset from 45 labelled 2D samples to over 100,000 1D profiles.

5.3.4 Neural network

A 1D convolutional network for image segmentation was created with the following structure. The network was created using stacked identical blocks. Each block contained a batch
Fig. 5.2 Comparison of old and new profile lines. Green lines show profiles extending from old white surface towards the old grey surface. Blue lines show profiles from the new expanded white surface towards the expanded grey surface. One noticeable feature (red arrow) is that the old white surface, extracted at lower resolution does not capture the full depth of the cortex, validating the decision to extend the profiles to resegment the pial and white surfaces at higher resolution. A second feature of note is that by smoothing the normal vectors, profiles are much better aligned with the probable path of the cortical column (orange box). Improved vertex-vertex correspondence is important for cytoarchitectural and even fMRI analyses, where summary statistics are treated as descriptors of a single cortical column, location or area but oblique sampling can unintentionally give rise to profiles crossing cytoarchitectonic borders or multiple columns with different functional properties. Finally the new surfaces have twice the resolution (four times the number of vertices) of the old surfaces, which will enable the layers to be smoother as the gaps between vertices is smaller.
Fig. 5.3 Extracting the training dataset. a) Two out of a total of 45 sample regions registered and combined with their corresponding 20$\mu$m BigBrain coronal section. Each layer has a given pixel value. b) Intensity profiles can be sampled from the same locations in the manual segmentation alongside the corresponding raw and anisotropically smoothed 20$\mu$m sections. Over 100,000 profiles were sampled from the 45 training regions to create a dataset with enough training examples for deep learning.

normalization layer to normalise feature distributions between training batches), a rectify non-linearity layer (used to model neurons which can have a graded positive (activation) but no negative response (Hahnloser et al., 2000) and a convolutional layer (Lecun et al., 1998). There was a final convolutional layer with filter size 1 and 7 feature maps, one for each cortical layer. A soft maximum was then applied to detect the most likely layer class for each pixel. The cost function was median class-frequency weighted cross-entropy. Class-frequency weighting was added to weigh errors according to the thickness of the layers so that incorrectly classified pixels in thinner layers were more heavily weighted than errors in incorrectly classified thicker layers (Eigen and Fergus, 2014). Raw and smoothed profiles were considered as two input channels, in a similar way to how colour images are divided into red, green and blue (RGB) channels. Each of these two channels provides important information. Anisotropically smoothed profiles contain contextual information, as smoothing was predominantly perpendicular to the profile direction, while raw 20$\mu$m profiles include fine-grained near-cellular scale local information. The network was trained on the 100,000 examples until the accuracy did not improve for 50 epochs (all 100,000 examples are seen once per epoch). At this point the previous best model was saved and used for subsequent testing on the full dataset. The trained network was then applied to profiles taken from across
the cortex for left and right hemispheres. The output was a vector of predicted layers for each of the 200 pixels, for each vertex on the cortical mesh.

5.3.5 Hyperparameter optimisation and cross-validation

There is no consensus method for finding optimum parameters for a neural network. Here a set of 50 experiments with random hyperparameters was carried out to explore their impact on training accuracy. Learning rate, convolutional filter size, number of layers (blocks), weight decay and batch size were all varied. For optimisation and cross validation the training segments were subdivided into 10 random subsets or folds. Initially 2 folds were removed from the dataset during training and hyperparameters were optimised for segmenting samples one of these folds. This fold was then included for training the optimised network to predict the accuracy of the final, previously unseen test region. This process was repeated 10 times to generate an estimate of the network’s ability to segment previously new cortical areas. Findings will not be reported here in detail but in summary the final network was initialised with 6 layers, filter size = 49, learning rate = 0.0005, weight decay = 0.001, where the learning rate determines the amount weights are updated on each iteration and weight decay determines the rate at that weights decrease each iteration which helps prevent overfitting.

5.3.6 Surface reconstruction: post-processing 1D profiles

1D classified profiles were transformed into mesh layer boundary reconstructions as follows. Transitions between predicted layers were identified for each profile and the coordinates of these transitions became vertex locations for the new layer meshes. For the small number of vertices where the network failed (less than 1%), vertex locations were interpolated from the neighbouring vertices to ensure a visually smoother surface. A small amount of geometric mesh smoothing was applied to the output surface (Taubin, 1995). This removed non-biologically high frequency changes in surface curvature, most commonly due to minor, local mis-registrations of consecutive 2D coronal sections. These smoothed surfaces can be used as registration targets for future iterations of the BigBrain volume.

5.4 Results

5.4.1 Neural network training

After around 250 epochs, the network approached a per-pixel accuracy of over 99% on over 90% of the training profiles. In the cross-validation, average accuracy on the test fold
was 86% suggesting the network was able to apply layer-specific features between different cortical areas. Nevertheless as this was not as high as overall training accuracy, there are differences between the laminar structure of certain cortical areas that make new areas more difficult to segment. Therefore, as not all areas were manually segmented visual inspection was carried out across other cortical areas.
Fig. 5.4 Cortical layers intersected on a 2D section with manually segmented layers. a) The boundaries clearly follow the same contours delineated by the manual segmentations, but appear to accurately follow the layer bounds outside of the training data. Higher resolution surfaces are able to follow layer contours more smoothly than the original cortical surfaces (For comparison, see Figures 4.7 & 4.2). b) At the V1-V2 boundary the thickness of layer IV changes dramatically in both manual and automated segmentations (between red and yellow lines), with additional peaks in V1 intensity due to the sublayers of layer IV. As each profile is individually segmented by the network, without reference to the neighbouring profiles, the network is able to apply area-specific rules according to the shape of the profile, suggesting it might be internally identifying the area from which the profile is extracted as being either V1 or V2.
5.4.2 Visual inspection of 3D layers of the cortex

Visually, automatically identified cortical layers closely follow bands of intensity within the BigBrain, continuing to follow the same features beyond the limits of training examples (Figures 5.1 & 5.4). The pial surface more closely follows the outer surface of the cortex than the original pial surface. This is partly due to the higher resolution mesh which enables a smoother surface and being extracted on 20µm data (the original was calculated on 200µm data). The layer I-II boundary is similarly improved relative to previous work, and more accurately follows the correct boundary in deep sulcal fundi. One clear difference is the white matter surface. In the original surfaces, vertices were placed at the maximum intensity gradient between gray matter and white matter, while the new white surface is based on the network trained on manual placement of the white boundary, which is defined by the presence of cortical neurons. On closer inspection, the maximum gradient appears to be at the border between sublayers VIa and VIb where the change in neuronal density is much sharper than at the boundary between white matter and layer VI (Figure 5.5). A second feature apparent on visual inspection is that the layers do not follow a single set of rules - they vary between cortical areas. This is most readily apparent at the V1-V2 boundary where layer IV is dramatically different (Figure 5.4b). Layer IV in is particularly thick in V1 has multiple sublayers creating extra peaks and troughs in the intensity profiles whereas in V2 it is much thinner. The transition from a thick layer IV to a thin layer IV occurs precisely at the boundary between these two regions suggesting the network is internally learning certain areal properties also. This property of 1D conv nets is now being explored explicitly in the classification of profiles into cortical areas.

5.5 Discussion

A convolutional neural network was developed to identify six cortical layers in 3D across the entire isocortex of the BigBrain (Figure 5.1). The segmented layers of the BigBrain provide cytoarchitectonic data on a previously unavailable scale. Being 3D, analyses are not restricted by the original plane of sections, and surface representation enables reanalysis of the cytoarchitectural data for any chosen parcellation scheme a large range of scales - from whole brain summary statistics down to each of over 1 million vertices. An important feature of this large amount of data is that it increases the ability to detect subtle effects that might not be evident in analyses of smaller scale cytoarchitectonic atlases. This affords opportunities to test many new and detailed hypotheses. An example, relating to in-depth analyses of layer thicknesses, is given in Chapter 6.
5.5 Discussion

Older white matter surface based on maximum intensity gradient
New white matter surface created by neural network learning from expert histological labelling

Fig. 5.5 Comparison of new and old white matter surfaces. For visual guidance the surfaces are overlaid on a 2D section, where manually segmented layers were available. The older white surface (green), which was identified on lower-resolution data and based on the location maximum intensity gradient. This surface was consistently superficial to the new surface (blue), which was created based on features derived from the histological definition of the white matter surface which is determined by the absence or presence of cortical neurons. This systematic difference highlights the importance of using histological expertise when translating across scales and fields to ensure consistent definitions. It also raises an important question on the placement of the white surface in MRI cortical reconstructions which is placed at the maximum MRI intensity gradient. This gradient is determined predominantly by myelin contrast, but the correspondence between MRI white surfaces and histological white surfaces remains to be tested.
The 3D layers of the BigBrain have applications for studies investigating the layered cortex in vivo. As current resolution and techniques cannot to segment layers from MRI images, they rely on prior models of the cortical layers - for example signal-source simulation in MEG (Troebinger et al., 2014) or for sampling laminar BOLD signal in fMRI (Muckli et al., 2015). This ground-truth, whole-brain histological models for areal layer depth, combined with a good understanding of how the layers vary with cortical morphology (Waehnert et al., 2014) will greatly improve the accuracy of existing models. These results also demonstrate the potential limitations of the standard neuroimaging approach of using solely intensity-based features to identify histological features such as the gray-white matter boundary. The original white matter surface was defined according to the standard MRI definition of maximum intensity gradient between grey matter and white matter (Fischl and Dale, 2000). However the neural network, trained on the histological delineation of the grey/white boundary, placed the surface consistently deeper and not at this maximum gradient, which likely corresponds to the layer VIa-VIb boundary (Figure 5.5). While this distinction might appear subtle, it highlights the importance of bringing to bear histological expertise. Moreover this raises an unexplored aspect of grey-white surface reconstructions in MRI, where the white matter surface is also placed at the maximum intensity gradient. As the source of contrast in MRI is predominantly due to myelin rather than cell bodies, the precise location of the maximum myelin gradient must be established in order to determine precisely what is being measured in MRI. It is possible that this is an example of a more general problematic assumption of direct and reliable correspondence between MRI-based findings and histological definitions. Such assumptions need to be validated.

The tools for profile-based analyses using 1D convolutional neural networks are being made freely available and have numerous applications beyond segmentation of layers in one BigBrain. Importantly, this trained network can now automatically segment cortical layers in 3D and as such can be applied to future 3D histological volumes. Additionally, it is not necessary to limit analyses to six cortical layers. For example, the same approach can be used for divisions of layers into sublayers, such as sublayers IVa, b, cα and cβ in V1 (Wagstyl et al., 2017). Moreover, in general, 2D stained sections of the cortex are still manually segmented. As such these tools are immediately transferable to automate this process. They can be applied to detect layers in other histological sections and with appropriate training data could be applied to any variety of preparations including myelin staining and autoreceptor radiographic images (Nieuwenhuys et al., 2015; Zilles et al., 1991).

An important future application of these tools is to the field of in vivo imaging. While the resolution of current MRI is not at 20µm, intensity profiles exhibiting layer-related features can now be extracted from in vivo MRI cortex (Fracasso et al., 2017, 2016). Already these
tools are able to identify 6 cortical layers on 20µm data, whereas human experts required 5µm resolution to see individual cells that indicate layers. This is therefore an example of supersampling, whereby higher resolution information is predicted from lower resolution images. Through downsampling the BigBrain and MRI simulation, future tests will be able to establish to what extent the position of cortical laminae can be inferred from structures measured using high resolution MRI. The vital input provided here is establishing a full ground truth laminar atlas against which predictions can be compared, and the profile-based tools with which these analyses could be carried out.

Finally profile-based segmentation addresses one of the fundamental challenges of deep learning in biomedical image analysis, namely the problem of expertly labelled sufficient examples with which to train the network. Here we created 45 training examples, manually annotating 8 2D sections from a total of 7400, which took a total of around 40 hours and represented less than 0.0001% of the total volume (Figure 5.3). The entire brain would therefore take over 45 years working round the clock for a single individual to manually annotate - automating segmentation was therefore essential. However training neural network to produce histologically valid cortical layers still required a large number of training examples. By creating thousands of profiles from each of these annotated segments hugely expanded the training sample. From the 45 initial examples, the full training dataset consisted of over 100,000 expertly labelled profiles from which the network could learn characteristic features of the cortical layers. These profile-based tools can be applied to a whole range of non-cortical questions, if they can be reposed as one of profile-based segmentation. One example is subcortical segmentation, such as transient developmental structures visible in foetal MRI ventricular and outer subventricular zones (Vasung et al., 2016), or delineation of subcortical abnormalities in strokes or epileptic lesions. Thus 1D convolutional neural networks for profiles segmentation have a wide range of neuroimaging applications beyond their original purpose of segmenting layers in the BigBrain.

5.5.1 Limitations

The major limitation of this approach as is that it is not possible to quantify the error in cortical areas for which there are currently no manually labelled training data. Visual inspection suggests that surfaces do follow features that appear to correspond to the correct cortical layers but the extent to which the network is generalisable to all unseen regions is unclear. In particular, frontal areas were less well represented in the training dataset than occipital, parietal and temporal areas which might affect layer accuracy in these areas. Nevertheless, should specific problem areas be identified, extra annotated regions can be added to the training dataset for future iterations of these surfaces.
5.5.2 Conclusions

In conclusion, I have created a fully segmented 3D model of 6 cortical layers in the BigBrain. The layers have the potential to further our understanding of large scale patterning in cortical microstructure, improved modelling of layers for macro-scale in vivo neuroimaging and a unique framework for bridging the gap between these two scales. Furthermore the convolutional neural network tools developed here for finding histological layers in 3D have wide-ranging applications including 2D microstructural studies and 3D in vivo cortical neuroimaging as well possibly much further.

5.5.3 Acknowledgements

I would like to thank the National Research Council of Canada for the development and customization of Atelier 3D for viewing the BigBrain. This research was enabled in part by support provided by Calcul Québec and Compute Canada. Parts of this research has received funding from the European Union's Horizon 2020 Framework Programme for Research and Innovation under Grant Agreement No 720270 (Human Brain Project SGA1). Parts of this research received funding from Neuroscience in Psychiatry Network, a strategic award by the Wellcome Trust (095844/Z/11/Z) and the MNI-Cambridge Collaboration Grant.
Chapter 6

Histological layer thicknesses in cortical hierarchies

6.1 Abstract

Cortical thickness, accessible through both in vivo and ex vivo neuroimaging techniques, is an informative marker of cortical structure. Initial in vivo structural MRI work has demonstrated that cortical thickness as a whole shows a transition or gradient across functional hierarchies. The question arises as to whether this is driven by an linear change across all layers or whether it is specifically driven by changes in thickness in particular layers across the hierarchy. I tested whether thickness gradients were present in histological thickness, measured in the BigBrain, and to what extent each layer exhibited a thickness gradient. Consistent with previous MRI thickness analyses, visual and somatosensory hierarchies exhibited cortical thickness gradients, with thickness increasing with hierarchical level. Critically, this overall gradient in thickness was driven by changes in specific supra and infragranular layers, predominantly layers III and V. No gradients of overall or layer thicknesses were found in the auditory hierarchy. Maps of histological and layer thicknesses provide a link between macroscale in vivo structural measures down to layer-specific and even cell-specific microstructural changes.

6.2 Introduction

Cortical thickness is a widely used marker of in vivo and ex vivo cortical structure. Early histological studies noted marked interareal thickness differences on post mortem histological sections (Brodmann, 1909; von Economo and Koskinas, 1925), which have since been
replicated (Fischl and Dale, 2000) and extended using in vivo MRI (Wagstyl et al., 2015) and alterations in this pattern may be seen in neuropsychiatric illness (Wagstyl et al., 2016). However the isocortex has six histologically defined layers, and it is unclear to what extent changes in cortical thickness are driven by different layers. Importantly, while some classical atlases of cortical layer thickness do exist, they are limited in the number of samples offered, the observer-dependence of their measurement and the inability to map the samples back to their original 3D location in the brain and reanalyse them with respect to known patterns of cortical organisation. I therefore sought to use the six isocortical layers segmented in the BigBrain to test whether gradients of cortical thickness measured using MRI are present in histological thickness and if so, which layers most strongly drive these changes.

MRI cortical thickness has demonstrated patterns of cortical thickness relating to functional and structural hierarchical organisation across the cortex of both macaque and humans (Wagstyl et al., 2015). However, while classical studies of cortical histology observed that primary sensory regions were thinner than their surrounding secondary sensory cortices (Brodmann, 1909; von Economo and Koskinas, 1925), the thickness gradients identified in MRI (Chapter 2) extended far beyond secondary areas into association cortical areas. Thus it is not known whether thickness gradients found in MRI are artefactual, driven by differences in cortical myelination causing systematic cortical reconstruction errors, or truly represent the underlying histology.

Uncovering how the organisational principles of cortical structure are related to function is crucial to understanding which aspects of cortical micro- and macro-structure contribute to functional differences. For instance, the fact that thickness gradients are inverse to neuronal density gradients demonstrates that increased neuropil and consequently intracortical connectivity match onto higher levels within the functional hierarchy (Wagstyl et al., 2015). However individual cortical layers are characterized by different neuronal morphologies and connectivity as well as differing roles in the functional microcircuit. Broadly speaking layer IV is the subcortical input layer, layers III pyramidal neurons are the source of feedforward cortico-cortical connectivity and layer V pyramidal neurons are the source of feedback connectivity (Bastos et al., 2012). As arborisation of these pyramidal neurons increases with hierarchical position, it is likely that these two layers are the primary drivers of increased cortical thickness. By establishing which layers are contributing to the overall cortical thickness gradients, it might even be possible to suggest which specific cells contribute most to these morphological differences and which aspects of the cortical microcircuit vary most with hierarchical function. Therefore establishing the histological source of MRI thickness gradients enables us to form links from macroscale morphology, through meso-scale layer thickness changes down to microscale inferences regarding changes in specific cell-types.
6.3 Methods

To date, gradients of histological cortical thickness in sensory hierarchies have not been explored. There may be several reasons for this. For instance the thickness gradient is likely to be subtle - in the MRI it was an increase of between 0.1 - 0.2mm per hierarchical level. Cortical morphology and oblique sectioning have a much larger effects on thickness measures (Figure 4.11) and these are difficult to account for without reconstructing the cortex in 3D and or making a large number of measurements. Furthermore 2D thickness measurements are done manually and are therefore time consuming and vulnerable to observer-dependent differences. Given the wealth of other informative histological measures such as neuronal morphology, receptor types, and connectivity, cortical thickness is perhaps not the most informative of microstructural measures. Nevertheless, cortical thickness and, with the advent of high-field higher resolution structural MRI, laminar thickness, offer an invaluable link between post-mortem histology and in vivo neuroimaging. Thus it is unclear whether MRI-based thickness gradients remained unobserved histologically because they are not present or have yet to be explored in sufficient detail.

The BigBrain can address many of these problems. First, it is 3D with complete cortical surface reconstructions. This enables measurement of total and individual layer thicknesses in 3D, measurement of geodesic distance across the surface and mapping of surface-based parcellation schemes. Second, automated segmentation of high-resolution surfaces creates over 500,000 sample points per hemisphere, affording statistical power to detect subtle changes hypothesised here.

I therefore sought to test the following two hypotheses of cortical laminar thickness using the automated segmentation of the cortex and its constituent layers in the BigBrain. First, I hypothesise that cortical hierarchies and geodesic distance predict increases in histological cortical thickness for sensory processing streams. Second I predict that thickness gradients are not uniformly exhibited by all layers, but are primarily driven by changes in layer III and V.

6.3 Methods

6.3.1 BigBrain cortical surface and layer reconstructions

3D reconstructions of six cortical layers, including the pial and layer VI-white matter boundary surfaces were created for the BigBrain. Please see chapters 4 and 5 for details of methods relating to the identification of cortical layers.
6.3.2 Thickness measurement

As in Chapter 2, thickness between two surfaces was measured as the average minimum distance between a vertex on one surface and the second surface (Fischl and Dale, 2000). Thickness maps were registered from the native CIVET surface to the CIVET symmetrical template surface for analysis with surface based parcellation schemes. Shrinkage due to the post-mortem fixation process was estimated in two ways - the first using the original brain-weight to estimate fresh volume and post sectioning volume (Amunts et al., 2005), the second taking the mean scale factor for the x, y and z components of the linear registration of BigBrain to the age-appropriate ADNI (Alzheimer’s Disease Neuroimaging Initiative) template brain. Both methods gave an estimated 83% shrinkage scale factor in each direction. In other words thickness measurements should be scaled up by 20% to give in vivo estimates. This correction factor was not applied to measurements made here as we are primarily interested in relative thicknesses, but should be considered when making comparisons with other datasets.

6.3.3 Mapping hierarchies

Hierarchies were mapped to the cortical surface by surface-based registration between the freesurfer average surface (fsaverage) (Fischl, 2012) and the CIVET resampled surface (Kim et al., 2005). The HCP Glasser atlas (Glasser et al., 2016) was mapped to the surface and regions were assigned levels in the visual cortical hierarchy by synthesising available macaque and human studies (Felleman and Van Essen, 1991; Grill-Spector and Malach, 2004; Grill-Spector et al., 2017; Markov et al., 2014). Auditory and somatosensory regions were taken from the HCP atlas but data were not available to assign these hierarchical positions. Instead, as in Chapter 2, geodesic distance was used as a surrogate measure of hierarchy to evaluate thickness gradients (Oligschläger et al., 2016; Wagstyl et al., 2015).

6.3.4 Geodesic distance

Geodesic distance from primary sensory areas was measured using the freely available surface distance function (https://github.com/tfunck/petco) (Funck et al., 2017). This was used to measure the shortest distance along the surface to each vertex from a given primary sensory area (visual, somatosensory and auditory).
Statistical analysis

Spearman’s rank correlation was used to evaluate the relationship between hierarchical levels and thickness measurements as the hierarchical levels are discontinuous rankings. Pearson’s correlation coefficient and linear model were used to evaluate the correlation between geodesic distance and thickness measurements. Confidence intervals for the Pearson’s correlation coefficients were calculated using the Fisher r-to-z transform. Confidence intervals were calculated according to the following equation.

\[ C.I. = \tanh \left( \text{arctanh} \left( r_s \right) \pm \frac{z}{\sqrt{N - 3}} \right) \] (6.1)

Here N is the number of sample points and z is determined by the chosen confidence level. For calculating 99% confidence intervals z was set at 2.58.

The linear model was used to quantify the magnitude of the relationship between layer thickness and distance, giving the estimated change in layer thickness per millimeter increase in distance from the primary sensory area. The extremely large number of vertices for each test causes even small effects statistically significant. As such correlation coefficients and β effect size were the more informative measures.

Results

Thickness maps

On visual inspection maps of BigBrain cortical thickness correspond with classical atlases of histological thickness reported by von Economo and Koskinas (Figure 6.1). In particular the motor cortex is the thickest part of the cortex with values over 3.8mm uncorrected (over 4.5mm when adjusted for shrinkage). The thickness of the motor cortex is often underestimated in MRI thickness measurement, probably due to the high degree of intracortical myelination which affects the grey-white contrast, causing the white matter surface to be too close to the grey surface, such that cortical thickness is underestimated (Glasser and Van Essen, 2011; Wagstyl et al., 2015). Clearly visible is the thin occipital cortex, where values fall below 2mm in the von Economo atlas and close to 1mm uncorrected (1.2 mm when adjusted for shrinkage). The new maps of histological thickness also offer hundreds of thousands of sample points, whereas the von Economo atlas offers a limited number of reported measurements.
Fig. 6.1 Cortical thickness The pattern of cortical thickness across the BigBrain (displayed on mid and expanded surfaces) matched that measured by von Economo and Koskinas. In particular the precentral gyrus, or primary motor cortex, which is often underestimated with MRI was the thickest area. Additionally the occipital cortex around the calcarine sulcus was particularly thin in both BigBrain and von Economo. BigBrain values have not been adjusted for shrinkage which was estimated at 20%. This would bring the maximum motor cortex thickness value to 4.5mm, within individual variability of the 4.8mm measured by von Economo.
Table 6.1 Results table of cortical thickness, laminar thicknesses and human visual hierarchy.

<table>
<thead>
<tr>
<th>Visual hierarchical level predicts cortical total and layer thicknesses</th>
<th>Spearman’s correlation and linear model effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visual cortex</td>
</tr>
<tr>
<td></td>
<td>$r_s$</td>
</tr>
<tr>
<td>Cortical thickness</td>
<td>0.59</td>
</tr>
<tr>
<td>Layer I</td>
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</tr>
<tr>
<td>Layer II</td>
<td>0.46</td>
</tr>
<tr>
<td>Layer III</td>
<td>0.44</td>
</tr>
<tr>
<td>Layer IV</td>
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</tr>
<tr>
<td>Layer V</td>
<td>0.62</td>
</tr>
<tr>
<td>Layer VI</td>
<td>0.54</td>
</tr>
</tbody>
</table>

6.4.2 Visual cortex

Cortical thickness was highly correlated with both hierarchical level and geodesic distance in the visual cortex (Figures 6.2 & 6.4, Tables 6.1 & 6.2). This was consistent with MRI-based findings derived in Chapter 2 (Figure 2.9). Cortical layers did not contribute equally to the total thickness gradient in the visual cortex (Tables 6.1 & 6.1). In rank correlations with hierarchical level, only layers II, III, V and VI showed significant correlations (Figure 6.3). However, when correlated with geodesic distance, all layers except Pearson’s R values greater than +/-0.35 (p-values were all significant, even on weak correlations due to the large number of vertices) (Figure 6.5). Layer IV thickness was negatively correlated with geodesic distance, while II,III, V and VI were all positively correlated. Layers III and V had the largest and most significant contributions to the total thickness gradient, followed by layer VI and then II. The negative layer IV gradient appeared to be primarily driven by the disproportionately thick layer IV in V1, with a much shallower slope further from the primary area.
Fig. 6.2 Cortical thickness strongly correlated with hierarchical position, based on fMRI studies of cortical hierarchy. This pattern was consistent across both hemispheres and matches the correlation found in Chapter 2 based on in vivo MRI cortical thickness.
Fig. 6.3 Supra and infragranular layers - II, III, V and VI - showed a strong correlation with hierarchical position. Layer I showed a weak positive correlation and layer IV showed no statistical relationship, although the layer IV was much thicker in V1 than in other visual regions. Correlation values displayed are Spearman’s rank correlations.
Table 6.2 Results table human sensory gradients.

Geodesic distance from primary sensory area predicts cortical total and layer thicknesses

<table>
<thead>
<tr>
<th></th>
<th>Cortical thickness</th>
<th>Layer I</th>
<th>Layer II</th>
<th>Layer III</th>
<th>Layer IV</th>
<th>Layer V</th>
<th>Layer VI</th>
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<tbody>
<tr>
<td><strong>Visual cortex</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>r</em></td>
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<td>0.18</td>
<td>0.37</td>
<td>0.43</td>
<td>-0.36</td>
<td>0.44</td>
<td>0.34</td>
</tr>
<tr>
<td><em>p</em></td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>99% C.I.</td>
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<td>[0.16,0.19]</td>
<td>[0.36,0.38]</td>
<td>[0.42,0.44]</td>
<td>[-0.37,-0.35]</td>
<td>[0.43,0.45]</td>
<td>[0.33,0.35]</td>
</tr>
<tr>
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<td>0.6x10^{-3}</td>
<td>1.9x10^{-3}</td>
<td>-1.5x10^{-3}</td>
<td>2.0x10^{-3}</td>
<td>1.5x10^{-3}</td>
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<tr>
<th></th>
<th>Cortical thickness</th>
<th>Layer I</th>
<th>Layer II</th>
<th>Layer III</th>
<th>Layer IV</th>
<th>Layer V</th>
<th>Layer VI</th>
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<tbody>
<tr>
<td><strong>Somatosensory cortex</strong></td>
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<tr>
<td><em>r</em></td>
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<td>0.16</td>
</tr>
<tr>
<td><em>p</em></td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>99% C.I.</td>
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<td>[0.04,0.07]</td>
<td>[0.1,0.13]</td>
<td>[0.34,0.36]</td>
<td>[0.10,0.13]</td>
<td>[0.21,0.24]</td>
<td>[0.14,0.17]</td>
</tr>
<tr>
<td>β</td>
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<td>0.2x10^{-3}</td>
<td>0.2x10^{-3}</td>
<td>2.3x10^{-3}</td>
<td>0.4x10^{-3}</td>
<td>1.5x10^{-3}</td>
<td>1.3x10^{-3}</td>
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<tr>
<th></th>
<th>Cortical thickness</th>
<th>Layer I</th>
<th>Layer II</th>
<th>Layer III</th>
<th>Layer IV</th>
<th>Layer V</th>
<th>Layer VI</th>
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<tbody>
<tr>
<td><strong>Auditory cortex</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>r</em></td>
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<tr>
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<td>0.7x10^{-3}</td>
<td>-0.7x10^{-3}</td>
<td>-2.7x10^{-3}</td>
</tr>
</tbody>
</table>
Fig. 6.4 Geodesic distance from V1, used as a surrogate marker of hierarchical position, was strongly correlated with cortical thickness. This pattern was consistent with that measured using *in vivo* MRI cortical thickness. Correlation values displayed are Pearson’s correlation coefficient and effect sizes were estimated using linear regression models.
Fig. 6.5 Similar to hierarchical position (Figure 3), layers II-III and V-VI showed a strong correlation with geodesic distance, with pyramidal layers III and V showing the largest effect sizes. The correlation with layer I was significant but the effect size was very small. Layer IV showed a negative correlation with geodesic, which was predominantly driven by the abnormally thick layer IV in V1, although there appears to be a small negative slope beyond V1. These patterns were extremely consistent across both hemispheres.
6.4 Results

6.4.3 Somatosensory cortex

Cortical thickness was highly correlated with geodesic distance in the somatosensory cortex (Figure 6.6, Table 6.2). This was consistent with MRI-based findings derived in Chapter 2 (Figure 2.8). Cortical layers did not contribute equally to the total thickness gradient in the somatosensory cortex (Table 6.2). The pattern for the somatosensory cortex was generally the same as for the visual cortex, albeit less significant. Only layer III thickness showed a correlation of greater than 0.35, although layers V and VI did also show an increase in thickness.
Histological layer thicknesses in cortical hierarchies

Fig. 6.6 Geodesic distance from S1, used as a surrogate marker of hierarchical position, was strongly correlated with cortical thickness. This pattern was consistent with that measured here in the visual cortex and in the somatosensory cortex using in vivo MRI cortical thickness in Chapter 2.
Fig. 6.7 Similar to hierarchical position (Figure 6.6), layers III and V showed the strongest effect size with geodesic distance, with smaller contributions from layer VI suggesting the same pyramidal cell layers are the primary drivers of the overall thickness gradient in the somatosensory cortex. The correlation with layers I, II, IV showed little thickness change with increasing geodesic distance. These patterns were extremely consistent across both hemispheres, and matched those found in the visual cortex.
6.4.4 Auditory cortex

Unlike the somatosensory and visual hierarchies cortical thickness was not correlated with geodesic distance in the auditory hierarchy (Figure 6.8, Table 6.2). This is also in contrast with MRI-based thickness findings, where there was a weak but significant correlation, which might now be attributable to cortical myelination (Glasser and Van Essen, 2011; Huntenburg et al., 2017). Similarly there was no convincing trend in any particular layer.

Fig. 6.8 Geodesic distance from A1, used as a surrogate marker of hierarchical position, was not correlated with cortical thickness. This pattern contrasts with those found in MRI and in the visual and somatosensory systems.
Fig. 6.9 There were no strong relationships between layer thickness and geodesic distance although there was a noticeable decrease in layer VI thickness. There was also significantly greater asymmetry in the findings. These differences may be due to actual biological asymmetries, but could be attributed to the damage in the temporal lobes caused during the brain extraction process.
6.4.5 Discussion

In this investigation I tested two main hypotheses. The first was that the cerebral cortex shows a gradient of increasing histological cortical thickness within sensory hierarchies. This was true of the visual and somatosensory hierarchies, but not the auditory hierarchy where no thickness gradient was found. The second was that individual cortical layers, particularly layers III and V would be the main drivers of these gradients. This was true of the visual and somatosensory hierarchies, with smaller but still significant contributions from layers II and VI. However, no laminar gradients were measured in the auditory hierarchy.

Gradients of thickness change are large scale markers of systematic changes in the cortical microcircuit. The volume of the cortex is 80-90% neuropil (Braitenberg and Schüz, 1991; Chklovskii et al., 2002), of which 60% is axons and dendrites and the remainder is synaptic boutons, spines and glia. As neuronal density decreases with increasing cortical thickness (Collins et al., 2010; la Fougère et al., 2011), and most of the volume of the cortex is neuropil, increased thickness is most likely to mark increased intracortical connectivity. At a laminar level, the strongest contributors to the overall thickness gradient were in layers III and V (Figures 6.5 & 6.7). Cell-morphological studies in macaques have shown that the cell size and dendritic arborisation of layer III and V pyramidal neurons increases across the visual pathway (Elston and Rosa, 1998, 2000; Scholtens et al., 2014). These cells play a particularly important role as the source of feedforward (layer III) and feedback (layer V) cortico-cortical connectivity (Markov et al., 2014). Similarly afferent axonal patch sizes scale with pyramidal neuronal arborization Lund et al. (1993). Increasing dendritic arborisation, axonal field size and numbers of synapses all contribute to an increase in the volume of laminar neuropil and are therefore the likely source of the layer thickness gradients measured here. Layers II and VI also increased in thickness in visual and, to a lesser extent, somatosensory pathways. However, while neurons located in these layers might exhibit increases their associated neuropil, the measured thickness change may in part be due to the extended arborisations of III/V pyramidal neurons forming connections within these layers. Therefore histological gradients of layer thickness provide us with a link between in vivo patterns of MRI cortical thickness and microstructural, neuron-specific changes in the cortical microcircuit where individual pyramidal neurons are forming more extensive and more numerous connections.

These patterns of structural changes were also correlated with visual hierarchical level as characterised by in vivo fMRI studies. Along these structural gradients, visual areas exhibit increasing temporal and spatial receptive fields as well as increasingly stimulus selectivity in functional response (Grill-Spector and Malach, 2004; Grill-Spector et al., 2017). Some of the receptive field properties may be predicted by the serial nature of a hierarchy, where if each neuron receives input from $x$ neurons in the previous layer, the receptive field size will
increase by a factor of $x$ with each hierarchical level. However the results here demonstrate that the cortical circuit exhibits intrinsic changes that facilitate these functional changes. Increased intracortical connectivity and neuronal structural receptive field - i.e. the number of unique inputs to each neuron - serve to increase the measured functional receptive field. Indeed studies of the primary visual cortex have demonstrated that thinner cortices lead to narrower functional receptive fields which directly impact a subjects’ performance in spatial discrimination tasks (Song et al., 2015). Further characterisation of interareal microstructural differences will reveal further insights into how differences in the cortical circuit give rise to functional specialisation.

While these sensory gradients have previously been characterised in vivo using MRI (Wagstyl et al., 2015), histological verification of these findings was essential as cortical thickness measured using MRI is known to underestimate the thickness of heavily myelinated areas (Glasser and Van Essen, 2011). Therefore these results verify that the histological thickness of the cortex is a major contributing factor to the gradients measured in the somatosensory and visual hierarchies. By contrast these findings contradict MRI thickness gradients found in the auditory (Wagstyl et al., 2015) and fronto-motor cortices (Thiebaut de Schotten et al., 2016). For the auditory cortex, cortical thickness and layer thicknesses showed no strong gradients. There are several possible reasons for this. The first is that the data are correct and the gradient of MRI thickness was driven by segmentation errors due to heavy myelination in the primary auditory cortex. The second is that a gradient is present, but was obfuscated by measurement errors particular to the BigBrain. In particular, these errors could be due to cuts in the temporal cortex, which might have affected both surface-registration of parcellation schemes and the accuracy of thickness measurements (see Limitations for a more detailed discussion). Finally, the auditory cortex exhibited the most subtle gradient of the three sensory pathways on MRI. In any case the results do not provide evidence supporting the existence of a strong histological thickness and laminar thickness gradient in the auditory cortex. This would suggest that there are fundamental organisational differences in auditory processing where there are consistencies between somatosensory and visual processing, but further validation of these findings is required before such conclusions could be drawn.

MRI-based gradients of cortical thickness have also been measured with thickness reportedly increasing from the primary motor cortex anteriorly to prefrontal regions (Thiebaut de Schotten et al., 2016). However histologically, the motor cortex was far thicker than prefrontal/orbito-frontal regions (Figure 6.1) and the frontal histological thickness gradient is likely in the opposite direction to that measured on MRI and to sensory thickness gradients. Unlike primary sensory areas, the primary motor cortex is thick, with large arborised
pyramidal neurons and extensive interneuronal connectivity (Lund et al., 1993; Mountcastle, 1997). Speculatively, this may be due to the increased amount of integration required for coordination between muscle groups for motor control, or the wide variety of direct cortico-cortical connections that terminate in the primary motor cortex, ranging from the primary somatosensory area to frontal and premotor areas. Regardless of the actual underlying microstructural cause and functional consequences of fronto-motor cortical neuroanatomy, meaningful conclusions can only be drawn from correct anatomical data.

6.4.6 Limitations

It is important to acknowledge that the gradients of laminar thickness measured may be affected by problems in the BigBrain dataset. The first limitation is that the post mortem brain was damaged during extraction and mounting. In some areas this resulted in minor shears. This problem was addressed to some extent through the utilisation of non-linear registration techniques. Nevertheless, some shifts in cortical tissue between consecutive sections are present and will affect the accuracy of layer reconstructions. In other areas the cortex has been torn. Smoothing and the large total number of sample points make it unlikely that these errors are affecting results. However, during brain extraction the saw entered the temporal lobes leading to a large cut which does cause problems as the surfaces currently follow the cut to some extent. This will cause local problems in the registration process and morphological measurement. A second limitation is that, without manually segmenting the entire cortex, it is not possible to know exactly how accurate layer measurements are in each region. However, in the 45 manually segmented regions, the automated laminar segmentations are exceptionally accurate and therefore it is unlikely that these errors are driving the large-scale trends of cytoarchitecture. Furthermore, these gradients were predicted by both MRI and histological studies.

6.4.7 Conclusions

Total cortical thickness and thicknesses for each of the six isocortical layers were measured in the BigBrain to explore the histological drivers of MRI-based thickness gradients. Overall the pattern of thickness in the BigBrain was consistent with histological atlases of cortical thickness. In the visual and somatosensory hierarchy a gradient of total thickness was present and was primarily driven by layers III and V, with smaller contributions from layers II and VI. In the auditory hierarchy no overall or laminar gradients were found. These findings provide a link between patterns of microstructural change and morphology measurable through MRI and emphasise the importance of testing MRI-based anatomical findings against ground-truth
histological techniques. The surface-based maps of total and layer thickness provide an invaluable tool for the neuroimaging community for validation and further characterisation of MRI thickness findings.
Chapter 7

Conclusions

7.1 Summary and implications

The goal of this thesis was to further our understanding of how morphological measures of the cortex, such as cortical thickness and curvature which are accessible through *in vivo* MRI, are related to microstructural, cytoarchitectural cortical properties. I also sought to explore ways in which these structural measures might be related to cortical function. This was achieved through establishing MRI cortical thickness as a cytoarchitecturally meaningful measure of structure, then developing the tools to identify the histological components of the cortex, i.e. its six layers, and analysing how the thicknesses of individual layers contribute to patterns of total thickness measured *in vivo*. Further analyses related patterns of cortical thickness change (and more precise understanding of how the thickness changes relate to underlying laminar change) to functional hierarchical organisation.

The first key insight in Chapter 2, based on structural MRI scans of both humans and macaque, was that the distribution of cortical thickness matched known patterns of cytoarchitectural and functional organisation. In particular sensory hierarchies exhibited a thickness gradient where sensory processing areas became increasingly thick with increasing hierarchical level. This was result was true of hierarchies in visual, somatosensory and auditory cortices and was present in all individuals measured. As neuronal density is inversely correlated with cortical thickness, these gradients were hypothesised to be caused by increases in neuropil rather than number of neurons. This suggested that increasing intracortical connectivity was a consistent structural change within sensory hierarchies. One further methodological advance was that geodesic distance was a useful surrogate of structural and functional hierarchical organisation, which is accessible in the absence of highly detailed anatomical data (Oligschläger et al., 2016; Wagstyl et al., 2015).
In Chapter 3, I showed that more detailed information can be gained from morphological markers if they are analysed and interpreted with reference underlying patterns of cytoarchitecture. For example, the patterns of laminar thicknesses with respect to gyri and sulci, or the distribution of layer thicknesses across the cortical areas can enable us to be more specific about what microstructural changes might be driving a morphological change (Wagstyl et al., 2016). These principles have since been applied in collaboration with other researchers from Neuroscience in Psychiatry Network (NSPN) to the analyses of cortical morphology and function in adolescent neuroimaging, where underlying cytoarchitectural subtypes strongly relate to patterns of cortical development (Vértes et al., 2016; Whitaker et al., 2016).

Although, Chapters 2 and 3 demonstrated that cytoarchitectural organisation as measured by classical atlases of histology can be immensely valuable for \textit{in vivo} morphological analyses, there are many limitations in generalising and mapping these century-old datasets to brain imaging data. These include limited numbers of samples and difficulty in co-registering existing samples to cortical surface reconstructions. Therefore, in Chapters 4, 5 and 6 automated tools for laminar analysis were developed in order to create more a detailed and comprehensive mapping of cortical cytoarchitecture.

In Chapter 4, I reported the development and testing of the tools for profile-based identification of 3D cortical layers in BigBrain. Initially I was only able to identify the layer I-II boundary and layer IV. Nevertheless this confirmed that the relative depths of cortical layers were closely related to cortical morphology. Layer IV was significantly correlated with cortical curvature (R=0.76, p<0.0001) such that layer IV was deeper in sulci and more superficial in gyri (Figure 7.1b). As well as confirming an underlying principle for interpreting the causes of gyral sulcal thickness changes applied in Chapter 3, the identification of the layer I-II boundary and layer IV has widespread implications for neuroimaging studies. Sampling intracortical MRI intensities has been utilised to investigate neurodevelopmental changes (Whitaker et al., 2016), pathological changes (Adler et al., 2017) and to create connectivity maps (Seidlitz et al., 2017). However, these studies sample MRI intensities at equal intracortical euclidean depths across gyri and sulci. This, in effect, means sampling different cortical layers in gyri versus sulci. When sampling a 3mm cortex with 1mm MRI voxels, the 30% relative depth change of layer IV in a gyrus vs a sulcus represents sampling data from entirely different voxels. Therefore this work stresses the importance of using cytoarchitectonically driven models of layer depths in MRI studies, such as the equivolumetric model (Waehnert et al., 2014). The profile-based approach also enabled quantification of the errors associated with 2D histological thickness measurement introduced by oblique sectioning of a curved cortex. This suggests there are significant limitations to measurements made in histological and pathohistological studies of 2D thicknesses.
Fig. 7.1 Diagramatic representation of links made between macroscale scale changes in total cortical thickness, mesoscale changes in laminar thickness and microscale changes in neuronal morphology. a) Gradients of increasing total cortical thickness through sensory hierarchies were driven primarily by layers III and V. Pyramidal neurons in these two layers increase in dendritic arborisation and size which contributes to overall increases in laminar volume. b) Cortical gyri were thicker than their adjacent sulci, with corresponding changes in laminar depth. Supragranular layers (blue) are thicker in sulci and infragranular layers (green) were thicker in gyri. The shearing and stretching of these layers due to cortical folding, which systematically alters their thickness, is also evident in changes to pyramidal neuron morphology. Sulcal pyramidal neurons have more tangentially oriented arbors, while gyral pyramidal neurons are more radially oriented. Changes in neuronal morphology directly affect neuronal electrophysiology and connectivity patterns and consequently microcircuit function.
As laminar intensity profiles extracted from different cortical regions varied significantly, more complex learning algorithms were required for segmentation of six cortical layers across the entire cortex. In Chapter 5, I used deep learning tools to segment 1D profiles of cortical intensity into six cortical layers. I trained a single 1D convolutional neural network to accurately segment profiles from 45 sample training areas. This trained classifier was able to generalise across the entire cortex to provide predicted locations of all six layers. The 3D, high-resolution surface reconstructions of cortical layers have many applications in neuroimaging. These were automatically registered to MRI surface templates including MNI152 and fsaverage and therefore maps of laminar thickness can be directly used as a cytoarchitectural resource for analyses like those in Chapters 2, 3 and 6. Thus any study with an MRI-derived region of interest can investigate the laminar structure of the corresponding region on BigBrain. Furthermore for modelling cortical layers in vivo, these six classifier-derived cortical layers provide the most complete structural model of histological layers, capturing both morphological and regional variability. Finally the profile-based approach addressed a fundamental problem in biomedical applications of deep learning - generating sufficient quantities of labelled training data. From just 45 2D examples I was able to generate over 100,000 intensity profiles, which enabled successful and generalisable training of the network.

In Chapter 6, 3D histological layers extracted from the BigBrain were used to examine patterns of cortical and laminar thickness. Initial inspection of the total cortical thickness map confirmed that, contrary to many MRI studies, the precentral gyrus or primary motor area is the thickest cortical area. As such, the thickness maps from structural MRI should be compared with a histological dataset like the BigBrain to explore precisely what MRI thickness signifies. I used these maps to verify that gradients of thickness in sensory hierarchies, first measured on MRI in Chapters 2 and 3 were present in the visual and somatosensory cortices but not in the auditory cortex. Within the visual and somatosensory hierarchies, gradients were driven primarily by increases in thickness of layers III and V, with lesser contributions from layers II and VI (Figure 7.1a). This is consistent with monkey studies of neuronal morphology that demonstrate increases in dendritic arborisation, number of spines, connectivity and overall volume of layer III and V pyramidal neurons through the visual processing pathways. This result therefore links large scale gradients of functional organisation and macroscale morphology, measured using in vivo MRI, with mesoscale changes in histological layer thickness and even cell-level changes in morphology and connectivity. This dataset now provides a framework for future analyses linking more detailed cytoarchitectural properties with higher-field, higher resolution in vivo neuroimaging.
7.2 Future work

The central aim of this thesis has been to link the macro-measures of morphology that are conveniently accessible through non-invasive neuroimaging to the micro-measures that more directly speak to cortical function and that more directly reflect the pathologies that we are trying to comprehend. Building on this work, I have begun several collaborative projects centred around this ambition.

7.2.1 Quantitative cytoarchitecture

The first area of future work is more detailed exploration of quantitative cortical microstructure. As demonstrated in this dissertation, cortical and laminar thicknesses create a set of links between in vivo structural and functional analyses and post mortem data. However, there are many aspects of functional specialisation for which we currently do not know the microstructural origin, such as fMRI temporal receptive field hierarchies (Grill-Spector et al., 2017). Some of these links might be provided by analyses of BigBrain sections at 1µm. Histological sections of BigBrain are being rescanned at 1µm in-plane resolution. From this higher resolution data it will be possible to identify, classify and measure individual cortical neurons, in other words it will be possible to obtain maps of neuronal density, neuronal subtypes and neuronal body orientation and size. Furthermore, other types of data such as autoreceptor radiography or neuronal transcriptomics can be registered more accurately to this high-resolution histological brain as a common microstructural template space before registration to MRI. Finding systematic patterns in these data will doubtless provide more insight into which microstructural properties are crucial for a variety of specialised cortical functions.

7.2.2 Cortical circuits and neural networks

Another area of future work is in the analysis of neural networks. In the work presented here, I have used neural networks and deep learning to aid neuroimaging analyses, but there is another area where neural networks can aid neuroscientific understanding. A neural net, trained to classify images or movies shows many parallels with the mammalian visual system. Successful image classifiers are hierarchically organised, with levels showing increasing spatial and temporal receptive fields similar to those seen in the visual pathways. For example, networks develop lower level nodes (or “neurons”) that act as edge detectors, and higher level neurons that show face selectivity (Kanwisher et al., 1997; Le et al., 2011). However the basic functionality of each non-linear computational node or neuron, unlike
its biological equivalent the cortical column, is normally identical at all levels. One way to aid our understand of systematically changing cortical circuits would be to investigate the activation properties of these nodes relative to their hierarchical position in the network or even carry out computational manipulations to determine the impact of implementing systematically varying microcircuits on classifier function. This is one of many potential cross-overs between deep learning research and neuroscience (Yamins and DiCarlo, 2016).

7.2.3 Cortical layers in vivo

A further area of future work is the development of tools to identify cortical layers in vivo. Chapter 5 demonstrates that neural networks are capable of accurately identifying cortical layers at 20µm, while histologists require 1-5µm data. BigBrain affords us the opportunity to experiment with the limits of this “supersampling” effect by further downsampling the profile data given to the network and testing how accurately the cortical layers can be predicted from lower resolution data. The current highest resolution for in vivo MRI is 250µm at which some laminar features are visible (Figure 4.3) (Lüsebrink et al., 2017), but it remains to be seen how accurately layer positions can be determined from these features. Crucially intracortical contrast for structural MRI is primarily driven by variations myelin and iron content, and therefore cortical intensity profiles do not necessarily exhibit features corresponding to histologically defined layers. An important step will therefore be translating the prediction of layers from histological profiles to MRI-based profiles. Simulating MRI from the BigBrain layers is one feasible option, but the ideal dataset would be a high-resolution MRI (preferably in vivo due to post mortem signal changes) acquired prior to post mortem sectioning and histological processing. From these data it might be possible to train a network to predict the location of histological layers from MRI features alone. Such an approach should enable us to directly measure interindividual, developmental and pathological laminar changes that can currently only be measured via indirect or non-specific approaches (Chapter 3, (Wagstyl et al., 2015; Whitaker et al., 2016)).

7.2.4 Cytoarchitectonice parcellation

A related avenue for future work is that of cortical parcellation. By accurately learning to identify cortical layers across a wide range of cortical areas (Chapters 4 and 5), the network has implicitly learned to apply different rules to different types of profiles, which almost certainly requires internal classification of profiles into different area-related classes. Intensity profiles have long been used to detect cytoarchitectonic borders (Schleicher et al., 1999), but currently they have not been used to classify profiles into specific areas. Accurately labelled
histological data from several post mortem brains would provide the ideal training dataset for adapting the 1D convolutional network approach to profile classification and therefore segmentation of cortical areas. Importantly the profile approach will enable training on 2D annotated sections and testing on 3D BigBrain. Such a parcellation would be an invaluable resource for exploring the links between cytoarchitectonic areas and cortical morphology (Fischl et al., 2008), and functional parcellation schemes (Glasser et al., 2016; Weiner et al., 2016), creating new possibilities for investigating microstructure and functional specialisation (Gomez et al., 2017).

### 7.2.5 Ongoing projects

There are number of projects that have emerged as a result of this thesis. These extend the presented work in the areas of machine learning, cytoarchitectural analysis and cortical structure in MRI. Here is a list of some of these projects and the groups involved:

1. Segmentation of cortical layers in the BigBrain and extension to MRI. Collaboration between Montreal Neurological Institute, Montreal Institute for Learning Algorithms, INM-1 Research Centre Jülich and University of Cambridge.

2. Classification of cortical areas in the JuBrain atlas. Collaboration between the Montreal Institute for Learning Algorithms, Montreal Neurological Institute, INM-1 at Julich Forszencentrum and University of Cambridge.


6. Automated analysis of BigBrain at 1µm. Collaboration between Montreal Neurological Institute, Croatian Institute for Brain Research, INM-1 at Research Centre Jülich and University of Cambridge.

The goal of this thesis was to create links between MRI and cortical cytoarchitecture. The five main chapters describe developments in using cytoarchitecture to understand MRI, and tools for automated analysis of histological data which can be used in conjunction with
MRI cortical surfaces. Through these advances I was able to link macroscale changes in cortical thickness, histological cortical layers and even neuron-specific changes in cortical microstructure. As a result of these developments, numerous new avenues for more detailed understanding of cortical structure have emerged and these are just beginning to be explored. The growing number of projects building on this work in machine learning, cytoarchitectural analyses and cytoarchitecturally motivated analyses of MRI demonstrate that the overall goal of this thesis has been achieved.
References


References


Eigen, D. and Fergus, R. (2014). Predicting depth, surface normals and semantic labels with a common Multi-Scale convolutional architecture.


References


Harlow, J. M. (1868). Recovery from the passage of an iron bar through the head. *Massachusetts Medical Society*.


Le, Q. V., Ranzato, M., Monga, R., Devin, M., Chen, K., Corrado, G. S., Dean, J., and Ng, A. Y. (2011). Building high-level features using large scale unsupervised learning.


R Core Team (2012). R: A language and environment for statistical computing.


References


References


y Cajal, S. R. (1899). *Comparative study of the sensory areas of the human cortex*.


