CHARACTERISING DISEASE-RELATED AND DEVELOPMENTAL CHANGES IN CORRELATION-DERIVED STRUCTURAL AND FUNCTIONAL BRAIN NETWORKS

František Váša

Supervisor: Prof. Edward T. Bullmore

Department of Psychiatry
University of Cambridge

This dissertation is submitted for the degree of
Doctor of Philosophy

Churchill College
July 2018
Babičce Janě, která mě naučila vždy sahat po vyšších větvích.
DECLARATION

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 and contains nothing which is the outcome of work done in collaboration with others, except as specified in the text and Acknowledgements. This dissertation contains fewer than 65,000 words including appendices, bibliography, footnotes, tables and equations and has fewer than 150 figures.

František Váša
July 2018
ACKNOWLEDGEMENTS

This document was typeset in \LaTeX{}, using the templates available at http://github.com/cambridge/thesis.

Work presented in Parts I and II is revised from material previously published in peer-reviewed journals. Experiments conducted within these studies (as well as in Part III), were principally designed and carried out by me. Contributions of all co-authors are listed below each reference.


Designed the study: PF, RJD, PBJ, IMG and ETB. Conceived and designed analyses: FV, JS, GR, OS and ETB. Processed and quality controlled data: KJW, RRG, FV and PEV. Conducted analyses: FV. Contributed analysis tools and code: FV, JS, RRG, KJW, PEV, MS and AAB. Wrote the article: FV and ETB. Critically appraised the article: all authors.


Conceived and designed analyses: FV, ETB, AXP. Processed and quality controlled data: AXP. Conducted analyses: FV. Wrote the manuscript: FV, AXP. Critically appraised the manuscript: FV, ETB, AXP.

Due to these contributions, work presented in Chapters 2-6 is presented in the plural form of a first-person narrative ("we"), while the Introduction and Summary (Chapters 1 and 7 respectively) are presented in the singular form ("I"). Please note that the above contributions do not affect the status of this thesis as being principally my own work.
I am very grateful to the Gates Cambridge Trust for kindly funding my PhD scholarship.

I am also grateful to the Gates Cambridge Trust, Churchill College Cambridge and the Guarantors of Brain for supporting my attendance at conferences throughout my thesis. Further, I am grateful to all participants in the COBRE (Center for Biomedical Research Excellence) and NSPN (Neuroscience in Psychiatry Network) studies included in this thesis. Finally, I would like to thank the Jana Kalousová Scholarship for additional personal support.

I am deeply grateful to Ed Bullmore for taking me on and supporting me throughout this fascinating period. Thank you, Ed. I am also indebted to Olaf Sporns for inviting me to Bloomington, for a stimulating and fun visit. My heartfelt gratitude goes to everyone I crossed paths with at the Brain Mapping Unit, and more generally everyone who has kindly collaborated with me, helped me in some way or simply cheered me on. In particular - Ameera, Gideon, Jakob, Kirstie, Manfred, Manuel, Max, Petra, Rafa, Sarah: thank you for your friendship, your help, and for standing by me through the ups and downs.

Merci, mes amis les plus propices, pour avoir toujours été là pour moi.

To my Sunnyside family: thank you for the love, the walks...and the thesis-fuelling roasts.

Mojí milované, vzácné a celkově báječné rodině: bez vás nic. Díky za všechno - jste nejlepší!

And to you, mine: thank you for everything.
Abstract

Human structural and functional brain architecture is increasingly studied by applying the mathematical framework of complex networks to data from magnetic resonance imaging. Connections (edges) in such brain networks are commonly constructed using correlations of features between pairs of brain regions, such as regional morphology (across participants) or neurophysiological time series (within participants). Subsequent analyses frequently focus on summary network statistics calculated using the strongest correlations, but often neglect potential underlying shifts within the correlation distribution. This thesis presents methods for the construction and analysis of correlation-derived structural and functional brain networks, focusing on the implications of changes within the correlation distribution.

First, schizophrenia is considered as an example disease which is known to present a reduction in mean correlation between regional neurophysiological time series. Previous studies reported increased network randomisation in schizophrenia, but these results may have been driven by inclusion of a greater number of noisy edges in patients’ networks, based on retention of a fixed proportion of the strongest edges during network thresholding. Here, a novel probabilistic thresholding procedure is applied, based on the realisation that the strongest edges are not necessarily most likely to be true following adjustment of edge probabilities for effects of participant in-scanner motion. Probabilistically thresholded functional networks show decreased randomness, and increased consistency across participants. Further, applying probabilistic thresholding eliminates increased network randomisation in schizophrenia, supporting the hypothesis that previously reported group differences originated in the application of standard thresholding approaches to patient networks with decreased functional correlations.

Subsequently, healthy adolescent development is studied, to help understand the frequent emergence of psychiatric disorders in this period. Importantly, both structural and functional brain networks undergo maturational shifts in correlation distribution over adolescence. Due to reliance of structural correlation networks on a group of subjects, previous studies of adolescent structural network development divided groups into discrete age-bins. Here, a novel sliding-window method is used to describe adolescent development of structural
correlation networks in a continuous manner. Moreover, networks are probabilistically thresholded by retaining edges that are most consistent across bootstrapped samples of participants, leading to clearer maturational trajectories. These structural networks show non-linear trajectories of adolescent development driven by changes in association cortical areas, compatible with a developmental process of pruning combined with consolidation of surviving connections. Robustness of the results is demonstrated using extensive sensitivity analyses.

Finally, adolescent developmental changes in functional network architecture are described, focusing on the characterisation of unthresholded (fully weighted) networks. The distribution of functional correlations presents a non-uniform shift over adolescence. Initially strong cortical connections to primary sensorimotor areas further strengthen into adulthood, whereas association cortical and subcortical edges undergo a subtler reorganisation of functional connectivity. Furthermore, individual subcortical regions show distinct maturational profiles. Patterning of maturation according to known functional systems is affirmed by partitioning regions developing at similar rates into maturational modules.

Taken together, this thesis comprises novel methods for the characterisation of disease-related and normative developmental changes in structural and functional correlation brain networks. These methods are generalizable to a wide range of scenarios, beyond the specific disease and developmental age-ranges presented herein.
# Table of Contents

List of figures .......................... xvii

List of tables .......................... xix

1 Background and Introduction .......... 1
   1.1 The brain as a complex network ................. 1
   1.2 Construction of human brain networks using magnetic resonance imaging .... 2
   1.3 Issues in mapping brain network architecture ................. 8
   1.4 Thesis structure .......................... 13

II Probabilistic thresholding of functional networks .......... 15

2 Probabilistic thresholding of functional (dys)connectomes in schizophrenia ............. 17
   2.1 Introduction .......................... 17
   2.2 Methods .......................... 18
      2.2.1 MRI data and processing .......................... 18
      2.2.2 Wavelet despiking and estimation of effective degrees of freedom ............. 19
      2.2.3 Graph construction .......................... 20
      2.2.4 Probabilistic thresholding methods .......................... 21
      2.2.5 Comparison of probabilistic and correlation-based thresholding ................. 23
      2.2.6 Sensitivity analyses .......................... 24
   2.3 Results .......................... 25
      2.3.1 Edge weight (correlation) distributions .......................... 25
      2.3.2 Properties of probabilistically-thresholded connectomes .......................... 26
      2.3.3 To what density can functional connectomes be built? .......................... 28
      2.3.4 Comparing r and P based thresholding .......................... 29
      2.3.5 Sensitivity analyses .......................... 32
   2.4 Discussion .......................... 37
2.4.1 Thresholding methods ........................................... 38
2.4.2 Further considerations .......................................... 39
2.4.3 Future work ....................................................... 40

3 Effects of probabilistic thresholding on randomisation in schizophrenia 43
3.1 Introduction ...................................................... 43
3.2 Methods ........................................................... 44
   3.2.1 MRI data and processing ...................................... 44
   3.2.2 Topology in networks probabilistically thresholded to fixed density . 45
   3.2.3 Topology in networks thresholded by probability or correlation ...... 46
3.3 Results ............................................................ 47
   3.3.1 Integration and segregation in probabilistically-thresholded networks 47
   3.3.2 Group differences in network disconnectivity and mean correlation . 50
   3.3.3 Differences in topology between r- and P-thresholded connectomes 53
3.4 Discussion .......................................................... 54
   3.4.1 Topological randomisation and schizophrenia ........................ 54
   3.4.2 Further considerations ......................................... 56
   3.4.3 Conclusion ..................................................... 57

II Sliding window construction of structural networks 59

4 Adolescent tuning of association cortex in structural brain networks 61
4.1 Introduction ........................................................ 61
4.2 Methods ........................................................... 63
   4.2.1 Participants .................................................... 63
   4.2.2 MRI acquisition and processing ............................... 63
   4.2.3 Rates of thinning and myelination ............................ 64
   4.2.4 Age-invariant structural network ............................... 64
   4.2.5 Development of age-resolved structural networks ................. 65
   4.2.6 Fitting and characterisation of developmental trajectories ........... 66
   4.2.7 Relationship of network development to age-invariant architecture . 68
   4.2.8 Spatial permutation test ....................................... 70
4.3 Results ............................................................ 72
   4.3.1 Age-invariant structural network ................................ 72
   4.3.2 Age-resolved structural networks ............................... 74
   4.3.3 Regional development of age-resolved structural networks .......... 76
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.4 Relationships of network development to thinning and myelination</td>
<td>76</td>
</tr>
<tr>
<td>4.3.5 Trajectories of Euclidean distance</td>
<td>80</td>
</tr>
<tr>
<td>4.3.6 Network changes in relation to the age-invariant network</td>
<td>81</td>
</tr>
<tr>
<td>4.4 Discussion</td>
<td>83</td>
</tr>
<tr>
<td>4.4.1 MRI studies of adolescent structural brain network development</td>
<td>84</td>
</tr>
<tr>
<td>4.4.2 Relationship to axo-synaptic connectivity (and its adolescent pruning)</td>
<td>86</td>
</tr>
<tr>
<td>4.4.3 Adolescent maturation of structural correlation and cortical structure</td>
<td>87</td>
</tr>
<tr>
<td>4.4.4 Further considerations</td>
<td>88</td>
</tr>
<tr>
<td>5 Robustness of sliding-window structural network analysis</td>
<td>89</td>
</tr>
<tr>
<td>5.1 Introduction</td>
<td>89</td>
</tr>
<tr>
<td>5.2 Methods</td>
<td>90</td>
</tr>
<tr>
<td>5.2.1 (In)dependence of results on sliding window parameters</td>
<td>90</td>
</tr>
<tr>
<td>5.2.2 Linear rates of change of node degree</td>
<td>90</td>
</tr>
<tr>
<td>5.2.3 Trajectories of structural correlation, covariance and variance</td>
<td>91</td>
</tr>
<tr>
<td>5.2.4 Effects of gender</td>
<td>91</td>
</tr>
<tr>
<td>5.2.5 Effects of regional reliability</td>
<td>91</td>
</tr>
<tr>
<td>5.2.6 Effects of irregularities in age-distribution of participants</td>
<td>92</td>
</tr>
<tr>
<td>5.2.7 Effects of non-linearities in trajectories of thinning and myelination</td>
<td>92</td>
</tr>
<tr>
<td>5.3 Results</td>
<td>93</td>
</tr>
<tr>
<td>5.3.1 (In)dependence of results on sliding window parameters</td>
<td>93</td>
</tr>
<tr>
<td>5.3.2 Linear rates of change of node degree</td>
<td>96</td>
</tr>
<tr>
<td>5.3.3 Trajectories of structural correlation, covariance and variance</td>
<td>96</td>
</tr>
<tr>
<td>5.3.4 Effects of gender</td>
<td>98</td>
</tr>
<tr>
<td>5.3.5 Effects of regional reliability</td>
<td>98</td>
</tr>
<tr>
<td>5.3.6 Effects of irregularities in age-distribution of participants</td>
<td>98</td>
</tr>
<tr>
<td>5.3.7 Effects of non-linearities in trajectories of thinning and myelination</td>
<td>100</td>
</tr>
<tr>
<td>5.4 Discussion</td>
<td>107</td>
</tr>
<tr>
<td>5.4.1 Parameters of the sliding window method</td>
<td>107</td>
</tr>
<tr>
<td>5.4.2 Properties of data used to construct the age-resolved network</td>
<td>108</td>
</tr>
<tr>
<td>5.4.3 Methodological considerations</td>
<td>109</td>
</tr>
<tr>
<td>5.4.4 Conclusion</td>
<td>109</td>
</tr>
</tbody>
</table>
### III Analysis of unthresholded functional networks

#### 6 Adolescent development of functional brain networks

6.1 Introduction ................................................................. 113
6.2 Methods ................................................................. 115
   6.2.1 Participants .................................................. 115
   6.2.2 MRI acquisition and processing ...................... 116
   6.2.3 Maturation of functional connectivity .......... 117
6.3 Results ................................................................. 120
   6.3.1 (In)dependence of functional connectivity on head motion .... 120
   6.3.2 Global maturation of functional connectivity .......... 122
   6.3.3 Maturation of functional connectivity in cytoarchitectonic classes . 124
   6.3.4 Nodal maturation of functional connectivity .......... 126
   6.3.5 Edge-wise maturation of functional connectivity .... 128
6.4 Discussion .............................................................. 134
   6.4.1 Impact of motion on estimates of functional network development ... 134
   6.4.2 Development of subcortico-cortical functional connectivity .. 135
   6.4.3 Adolescent maturation is patterned by known intrinsic networks ... 137
   6.4.4 Limitations and future work ......................... 137
   6.4.5 Conclusion .................................................. 138

#### 7 Summary and concluding remarks

7.1 Summary .............................................................. 139
7.2 Convergent themes ................................................. 141
   7.2.1 Correlation-derived brain networks ................. 141
   7.2.2 Adolescent development of structural and functional brain networks 142
   7.2.3 Development and disease .............................. 143
7.3 Generalisability of contributions .......................... 144
   7.3.1 Probabilistic thresholding .......................... 144
   7.3.2 Sliding window methods ................................ 145
   7.3.3 Characterisation of unthresholded networks ....... 146
7.4 Recommendations .................................................. 146
7.5 Future directions ................................................ 147
Table of contents

References 151
Appendix A  Overview of literature on adolescent brain network development 173
Appendix B  Availability of data and code 179
Acronyms 181
# List of Figures

1.1 Construction and analysis of brain networks using MRI. ........................................ 3  
2.1 Methods of probabilistic graph construction and analysis. ................................. 21  
2.2 Impact of adjustment of P values for effective df. ............................................... 25  
2.3 Decreased (unthresholded) correlations in patients with schizophrenia. .......... 26  
2.4 P-thresholded connectomes are more disconnected in schizophrenia. ............ 27  
2.5 Nodal degree and modular organisation of P value thresholded networks. ....... 28  
2.6 Thresholding connectomes to fixed edge density (based on P values). .............. 29  
2.7 Proportion of edges that differ between r- and P-thresholding. ......................... 30  
2.8 P-thresholded connectomes show increased edge consistency. ......................... 31  
2.9 Independence of results on motion. ................................................................. 33  
2.10 Negative edges in networks thresholded in order of increasing P-value. ......... 34  

3.1 Effects of non-significant edges on global efficiency. ......................................... 48  
3.2 Effects of non-significant edges on transitivity. ............................................... 49  
3.3 Effects of non-significant edges on connected components. ............................ 51  
3.4 Mean correlation in subsets of significant and non-significant participants. ........ 52  
3.5 Differences in topology between r- and P-thresholding. .................................... 53  

4.1 Construction of age-invariant and age-resolved structural correlation networks. 67  
4.2 Surface maps for two independent community templates. ............................... 69  
4.3 Demonstration of the spatial permutation test. ............................................... 71  
4.4 Partitions of the age-invariant structural correlation network into modules. ....... 73  
4.5 Global trajectories of age-resolved structural correlation networks. ................ 75  
4.6 Regional development of structural correlation networks. .............................. 77  
4.7 Rates of thinning and myelination and derived results. .................................... 78  
4.8 Relationship of maturation of cortical morphology and structural networks. ...... 79  
4.9 Trajectories of Euclidean distance spanned by retained edges. ......................... 80  
4.10 Development of structural networks in relation to human brain communities. .... 82
5.1 (In)dependence of results on sliding window parameters. 95
5.2 Fitting linear models to measure change with age 96
5.3 Decomposition of structural correlation. 97
5.4 Effects of regional reliability on trajectories of structural correlations. 99
5.5 Effects of inhomogeneity in the age-distribution of participants. 101
5.6 Effects of regional reliability on trajectories of structural correlations. 103
5.7 Effects of subtle non-linearities in myelination trajectories. 104
5.8 Rates of thinning and myelination in sub-samples of participants. 106
6.1 Effects of head motion on functional connectivity. 121
6.2 Global trajectories of the correlation distribution. 123
6.3 Higher percentiles of the correlation distribution strengthen faster. 123
6.4 Maturation of functional connectivity within cytoarchitectonic classes. 125
6.5 Regional maturation of functional networks. 126
6.6 Maturation of individual subcortical regions. 130
6.7 Relationships between edge-wise maps within cytoarchitectonic classes. 131
6.8 Communities of edge-wise linear models. 133
# List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>(In)dependence of main results on age and gender.</td>
<td>36</td>
</tr>
<tr>
<td>2.2</td>
<td>Consistency of results across wavelet scales.</td>
<td>36</td>
</tr>
<tr>
<td>2.3</td>
<td>Consistency of results across thresholds of edge significance.</td>
<td>37</td>
</tr>
<tr>
<td>5.1</td>
<td>(In)dependence of results on sliding window parameters.</td>
<td>94</td>
</tr>
<tr>
<td>6.1</td>
<td>Frequency and significance of changes in subcortico-cortical connectivity.</td>
<td>129</td>
</tr>
<tr>
<td>A.1</td>
<td>Adolescent development of structural correlation networks.</td>
<td>175</td>
</tr>
<tr>
<td>A.2</td>
<td>Adolescent development of whole-brain resting-state functional networks.</td>
<td>177</td>
</tr>
<tr>
<td>A.3</td>
<td>Adolescent development of subcortical resting-state functional networks.</td>
<td>178</td>
</tr>
</tbody>
</table>
CHAPTER 1

BACKGROUND AND INTRODUCTION

1.1 The brain as a complex network

It has long been suspected that complex brain functions emerge from interactions of distributed neural elements, based on the work of pioneering anatomists such as Theodor Meynert (1833-1892), Carl Wernicke (1848-1905) and Santiago Ramón y Cajal (1852-1934). At the microscopic scale, this distributed substrate is formed by neurons, interconnected by axons and dendrites. At a larger scale populations of neuronal bodies form the brain’s grey matter, while bundles of axons form the white matter pathways subserving communication between grey matter regions. Recently, the advent of magnetic resonance imaging (MRI; Lauterbur, 1973) enabled non-invasive and harmless mapping of brain structure and function (Bandettini et al., 1992; Ogawa et al., 1992; Kwong et al., 1992), substantially deepening our understanding of human brain anatomy and physiology. Early studies confirmed the distributed nature of neural systems, and their role in behaviour, cognition and disease (Raichle, 2009).

A simultaneous emergence of complex network mathematics in the form of graph theory enabled formal characterisation of networks. Since Leonhard Euler’s (1707-1783) early identification of the concept of network topology, the more recent ubiquitous presence of interconnected systems led to the emergence of new quantitative graph-theoretical measures (e.g.: Watts and Strogatz, 1998; Latora and Marchiori, 2001). Initial pioneering studies (Sporns et al., 2000; Hilgetag et al., 2000) applied such measures to tract-tracing data from the macaque (Felleman and Van Essen, 1991) and the cat (Young, 1992). Subsequent studies applied graph-theoretical analysis to human brain networks or "connectomes" (Sporns et al., 2005; Hagmann, 2005), including both functional networks constructed from magnetoencephalography (Stam, 2004) and functional MRI (Salvador et al., 2005a; Achard et al.,...
2006), as well as structural networks from diffusion-weighted MRI (Hagmann et al., 2007, 2008; Iturria-Medina et al., 2007) and structural MRI (He et al., 2007).

In this chapter, I will first briefly describe the primary steps involved in the construction and analysis of human structural and functional brain networks. Subsequently, I will highlight some of the outstanding issues in the mapping of brain network architecture. While much within these sections applies across methods for network construction and analysis, emphasis will be placed on structural and functional networks constructed using correlations. Finally, I will summarise the structure of this thesis.

### 1.2 Construction of human brain networks using magnetic resonance imaging

This thesis focuses on networks constructed using MRI. Structural MRI (sMRI; Lerch et al., 2017) and diffusion weighted imaging (DWI; Hagmann et al., 2006) are standard approaches to noninvasively map human neuroanatomy, while functional MRI (fMRI) offers a combination of high spatial and temporal resolution to map human neurophysiology (Sejnowski et al., 2014). Note that other methods exist to map human brain network organisation; for example, functional networks can be constructed using electro- or magneto-encephalography (EEG or MEG). Many of the methods presented in this thesis in the context of fMRI are also potentially applicable to EEG and MEG.

Moreover, a range of methods exists to construct brain networks using MRI data: seed-based methods, which yield a map of voxels (or vertices) connected to a chosen "seed" voxel (or vertex) of interest (e.g.: Lerch et al., 2006), multivariate dimensionality reduction methods such as principal or independent component analysis (PCA or ICA), yielding networks of voxels (or vertices) that are mutually independent in space or time (e.g.: Beckmann et al., 2005), as well as graph-theoretical methods, relying on estimates of connectivity between all pairs of a set of regions of interest (Fornito et al., 2016). This introduction and this thesis focus on the latter.

Construction of brain networks varies based on the type of network (structural or functional) and based on the specific imaging modality used (e.g.: structural or diffusion MRI); however, all construction pipelines involve a common set of steps (Fig. 1.1). These are: (i) acquisition of MRI images (and initial pre-processing), (ii) parcellation of grey matter into regions of interest (network nodes), (iii) estimation of association between pairs of brain regions (network edges) and, optionally, (iv) thresholding to retain a subset of connections. Subsequently, (v) networks can be analysed using graph-theoretical methods.
Construction of human brain networks using magnetic resonance imaging

1.2 Construction of human brain networks using magnetic resonance imaging

Fig. 1.1 Construction and analysis of brain networks using MRI. (i) Different imaging data may be used to construct brain networks, including diffusion, structural and functional MRI. (ii) Following standard pre-processing steps, grey matter is parcellated into regions, which correspond to network nodes. The parcellation is applied to the structural ($T_1$) scan, before being registered to the other modalities. (iii) The definition of edges is modality-specific. In the case of diffusion imaging, edges correspond to white matter tracts, whose trajectories are reconstructed from local estimates of fiber orientation through computational tractography. In the case of structural (covariance) and functional networks, edges correspond to statistical associations; in structural networks, these generally capture co-variation of morphology (such as cortical thickness) between pairs of regions across subjects, while in functional networks these are within-subject relationships between pairs of regional BOLD time series. Measures of association used can be marginal (yielding all-to-all connectivity) or partial (capturing only direct interactions), as well as (in the case of functional networks) symmetric or asymmetric (yielding respectively undirected and directed networks). (iv) Following estimation of connectivity, networks may be thresholded to retain only a subset of (generally the strongest) connections. (v) Finally, networks may be characterised using a range of graph-theoretical measures, capturing architectural features such as highly connected hubs, paths or communities of densely inter-connected nodes.
i) (Magnetic Resonance) Imaging

Different imaging sequences and/or modalities may be used to construct brain networks, capturing different aspects of brain connectivity.

The standard sequence used to obtain a structural image of the brain is the $T_1$-weighted scan, in which tissue contrast is determined by factors such as concentration of macromolecules and water content (Lerch et al., 2017). Moreover, structural scans such as $T_1$ are generally required to register individual scans to a template, to segment the brain into grey and white matter and to parcellate grey matter into regions of interest, regardless of the type of brain network being constructed.

Diffusion imaging relies on the estimation of the rate of water diffusion, following the application of diffusion encoding gradients (which may be of varying strength) in a number of directions. Water diffusion within each voxel (volume pixel) will depend on the its microstructural organisation - for example, water will diffuse more readily in parallel to a population of white matter fibers than perpendicular to it (Hagmann et al., 2006).

Finally, functional MRI relies on the differential magnetic susceptibility of oxygenated and deoxygenated blood to measure changes in blood oxygenation (yielding the blood-oxygenation-level-dependent or BOLD signal). Due to the fact that grey matter areas (or neurons) which are more active consume more oxygen, the BOLD signal provides an indirect estimate of neural activity (Logothetis and Wandell, 2004).

Prior to the parcellation of data and subsequent estimation of connectivity, numerous (pre-)processing steps are applied, including the realignment of volumes, brain extraction (from the skull) and correction of motion artefacts, among others. As these steps are not specific to network analysis of MRI data, they are not described in detail here; instead, where relevant, they are discussed further below and throughout the thesis.

ii) Parcellation

Nodes of the macroscopic brain network correspond to regions of grey matter, which can be parcellated according to different criteria. Previous efforts to define cortical parcellations have included purely anatomical parcellations with boundaries lying along sulci (Tzourio-Mazoyer et al., 2002; Desikan et al., 2006), functional parcellations using fMRI data to identify regions exhibiting homogeneous BOLD signal (Yeo et al., 2011; Craddock et al., 2012; Joliot et al., 2015), or parcellations combining data from multiple MRI modalities (Glasser et al., 2016). Other efforts have included parcellations based on the digitisation (Scholtens et al., 2016) of a pioneering cytoarchitectonic atlas (von Economo and Koskinas,
1.2 Construction of human brain networks using magnetic resonance imaging

1925), or integration of histological, chemoarchitectonic and neuroimaging data (Ding et al., 2016).

Furthermore, principled parcellations presenting large variability in region size are often sub-parcellated into regions of more uniform surface area or volume (Cammoun et al., 2012; Romero-Garcia et al., 2012), to ensure that nodal importance may (insofar as possible) be determined by a region’s connectivity rather than its size.

iii) Connectivity

Following the acquisition of images and the definition of network nodes, associations between pairs of nodes are estimated, which correspond to network connections or edges.

One method for constructing structural brain networks, based on the observation that brain structure co-varies across individuals, is to evaluate statistical relationships of morphological properties between pairs of regions across a group of participants (Alexander-Bloch et al., 2013a; Evans, 2013). The structural features most commonly used to construct these "structural covariance" networks are cortical thickness, grey matter volume and surface area, although others have been used as well (e.g.: gyrification; Palaniyappan et al., 2015). Unsurprisingly, different morphometric features yield different structural network architectures (Sanabria-Diaz et al., 2010; Yang et al., 2016).

Beyond the (historically more popular) estimation of morphological relationships across a group of participants, it has recently been proposed that covariation of morphology can also be estimated within individuals, by quantifying the similarity of voxels within local neighbourhoods (Tijms et al., 2012) or regions of interest (Kong et al., 2014, 2015), or by comparing a range of multi-modal morphometric features (Seidlitz et al., 2018).

Individual structural networks can also be constructed from DWI data using computational tractography, where edges correspond to trajectories of axonal pathways between pairs of regions estimated from voxel-wise orientation of white matter (Jbabdi et al., 2015). Studies carried out within this thesis focus on other methods for construction and analysis of brain networks, due in part to known limitations of tractography (Thomas et al., 2014; Reveley et al., 2015), including detection of a large proportion of false positive connections (Maier-Hein et al., 2017).

Finally, in functional networks, edges correspond to measures of statistical association between regional time series (van den Heuvel and Hulshoff Pol, 2010). These associations may be computed using a range of statistical measures.
Measures of statistical association

Measures of statistical association used to construct brain networks can be distinguished into several categories.

The first distinction is whether the statistical measure is symmetric, thus yielding undirected associations, or whether it is asymmetric, yielding directed associations. Of note, the calculation of associations between morphological features can only yield symmetric structural networks; however, when computing associations between time series in functional networks, the temporal sequence of the signal can (under certain circumstances) be leveraged to derive directed interactions. A second distinction is whether both direct and indirect associations are estimated, using marginal measures, or whether the influence of indirect interactions (such as input into two nodes from a common source) is removed using partial measures. Marginal measures yield fully weighted networks where non-zero associations are estimated between all pairs of regions; partial methods yield sparse networks, where some pairs of regions do not directly interact.

The most popular measures of statistical association between variables used to construct edges in brain networks are symmetric and marginal, yielding undirected and fully weighted networks. This family of measures includes the correlation coefficient, by far the most popular measure of network association. Generally, the Pearson’s correlation coefficient \(r\) is used, which assumes a linear relationship between variables:

\[
r_{xy} = \text{corr}(x, y) = \frac{\text{cov}(x, y)}{\sigma_x \sigma_y} = \frac{\sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2 (y_i - \bar{y})^2}}
\]

where \(\text{cov}(x, y)\) refers to the covariance between variables \(x\) and \(y\), \(\sigma_x\) and \(\sigma_y\) refer to their (sample) standard deviation, and \(\bar{x}\) and \(\bar{y}\) refer to their means. Notably, the Pearson’s correlation coefficient only captures the extent to which two variables are linearly related. Other variants of the correlation coefficient may be used as well, such as the non-parametric Spearman’s \(\rho\) or Kendall’s \(\tau\), which both measure agreement between rank ordering of the input variables - and will thus lead to high absolute values of correlation even for potentially non-linear (but monotonic) relationships. All three variants of the correlation coefficient lie between -1 and 1.

Another popular symmetric marginal measure is the mutual information, which captures the information shared by two variables, including both the linear and non-linear dependence between them. It can be expressed in terms of the entropy \(H\), a measure of the amount of information (or uncertainty) associated with a variable:
\[ I(X;Y) = H(X) + H(Y) - H(X,Y) = H(X) - H(X|Y) = H(Y) - H(Y|X) \quad (1.2) \]

where \( H(X) \) (or \( H(Y) \)) is the marginal entropy, a measure of uncertainty about \( X \) (or \( Y \)), \( H(X,Y) \) is the joint entropy, capturing uncertainty associated with both \( X \) and \( Y \), and \( H(Y|X) \) (or \( H(X|Y) \)) is the conditional entropy of \( Y \) conditioned on \( X \) (or \( X \) conditioned on \( Y \)), which is a measure of information about \( Y \) not contained within \( X \) (and vice-versa).

Note that in the case where the joint distribution for \( X \) and \( Y \) is a bivariate normal distribution (meaning that both marginal distributions are normal), the mutual information shared by \( X \) and \( Y \) is uniquely determined by their correlation. Hlinka et al. (2011) have investigated the suitability of the (linear) correlation coefficient as an undirected measure of (instantaneous) association between fMRI time series, reporting that only approximately 5% of the mutual information between time series (which are almost Gaussian) is neglected by the linear correlation coefficient.

Other symmetric marginal measures have been used to construct functional (and in some cases structural) networks as well, including the coherence (Sun et al., 2004), \( h^2 \) (Wang et al., 2014) and multivariate distance correlation (Geerligs et al., 2016).

Partial analogues exist for many of the above measures, including the partial correlation and the partial mutual information, both of which have been used to construct brain networks (e.g.: by Liu et al. (2008) and Salvador et al. (2005b) respectively). While these measures provide, in theory, a more "pure" estimate of connectivity by only capturing direct interactions, their application in practice is difficult. To enable controlling for the effects of all remaining regions when estimating a direct pairwise association, these methods require a far larger number of samples (e.g.: time-points for functional networks) than the number of regions - a criterion that is difficult to satisfy using standard MRI data (although it is becoming more accessible with longer acquisition times in selected datasets, e.g.: Gordon et al. (2017)).

Moreover, while directed analogues for many of these measures have been defined (Wang et al., 2014), more popular directed measures include transfer entropy and granger causality, both of which use past values of a time series to predict future values of another time series (Seth et al., 2015).

iv) Thresholding

Following the estimation of associations between pairs of nodes, many analyses focus on only a subset of edges. The elimination of a set of edges is called thresholding, and is most
commonly applied following estimation of connectivity using marginal association measures or probabilistic tractography, both of which yield dense (all-to-all connected) networks. However, one may wish to threshold sparse networks, as obtained for example from partial association measures or deterministic diffusion tractography algorithms, as well.

By far the most common form of thresholding retains edges according to weight, thus retaining the strongest edges. Weight-based thresholding can be applied either by retaining edges surpassing a certain weight (absolute thresholding), or retaining a fixed proportion of the strongest edges in each participant (proportional thresholding) (Fornito et al., 2013).

There are several justifications for thresholding. One is the assumption that the strongest edges are the most relevant and most likely to represent signal. Another motivation is that many measures of network organisation are not applicable to negative edges (Schwarz and McGonigle, 2011), present when edge-wise associations are quantified using measures such as the correlation coefficient. Finally, some network measures are only applicable to sparse graphs. (Although see more on these points below.)

v) **Graph-theoretical analysis of brain networks**

Finally, the network can be characterised using a range of graph-theoretical measures (Rubinov and Sporns, 2010). Many of these measures are applicable to unthresholded, fully weighted networks (Rubinov and Sporns, 2011), although the presence of negative edges in correlation-based networks (Schwarz and McGonigle, 2011) precludes the application of certain topological measures (such as measures based on path length).

A wide range of network measures are available to characterise complex networks. These include global network measures which provide a single summary statistic per network, mesoscale network measures describing subnetworks such as modules (Sporns and Betzel, 2016) or rich-clubs (van den Heuvel and Sporns, 2011) as well as local measures which provide a summary statistic for each node, for example enabling the identification of influential "hub" nodes (Sporns et al., 2007). Many of these measures relate to two key features of network organisation: segregation, indicative of the presence of densely connected groups of nodes, and integration, corresponding to relatively short topological distances between any pair of nodes (even in relatively large networks).

### 1.3 Issues in mapping brain network architecture

Although construction and analysis of human brain networks using data from MRI is now an established practice, there are numerous outstanding issues. Below, some of these questions are discussed, in order of the construction and analysis pipeline summarised above.
Effects of motion (and other artefacts) on network architecture

One issue that has persistently been resurfing, despite having been declared as resolved multiple times, is participant in-scanner motion. This was recognized as an issue in fMRI long ago (Hajnal et al., 1994; Friston et al., 1996), before more recently being acknowledged as problematic in diffusion imaging (Yendiki et al., 2014) and finally sMRI (Reuter et al., 2015; Alexander-Bloch et al., 2016; Savalia et al., 2017) as well. In fMRI, motion has complex effects on the signal - including increases, decreases or complex waveforms, depending on factors such as the timing, duration and trajectory of motion (Power et al., 2015). Crucially, motion can substantially affect estimates of functional connectivity (Power et al., 2012; Satterthwaite et al., 2012). Initial motion-correction approaches involved regression of motion parameters and their derivatives from voxel-wise BOLD time series (Friston et al., 1996), or regression of the average or "global" signal (Aguirre et al., 1998; discussed further below). Later solutions included censoring of motion-affected frames (Power et al., 2012), removal of non-BOLD (artefactual) signal using independent component analysis applied to multi-echo fMRI data, based on the dependence of the BOLD signal on echo time (Kundu et al., 2012, 2013), or "despiking" of motion-related non-stationary events from a wavelet decomposition of the signal (Patel et al., 2014). The latter method was subsequently extended to provide revised estimates of voxel-wise effective degrees of freedom ($df$) of the BOLD time series, which due to denoising are lower than the nominal $N(df) = N$(time-points), and which affect estimates of edge probability when incorporated into network analysis (Patel and Bullmore, 2016).

Other prominent artefacts, particularly in fMRI, relate to respiration, vasculature and arousal (e.g.: Murphy et al., 2013; Caballero-Gaudes and Reynolds, 2017).

One approach proposed to "clean" fMRI time series, suggested to apply regardless of the origin of the artefacts (motion, respiration, vasculature) is regression of the brain-wide average or "global" signal from voxel-wise time series (Aguirre et al., 1998; Murphy and Fox, 2017). Initially suggested in the context of task-based fMRI, global signal regression (GSR) became particularly controversial in the context of the resting-state fMRI literature, due to its effects such as zero-centering of the correlation distribution and the (disputed) introduction or inflation of anti-correlations between cognitive subnetworks (Fox et al., 2005, 2009; Murphy et al., 2009). Recent commentaries and studies have reported that the distortion of the correlation structure by GSR depends on the dimensionality of the underlying fMRI signal, which is unclear (Power et al., 2017b; Uddin, 2017; Power et al., 2017a). GSR has been investigated in the context of graph-theoretical analyses of fMRI-derived connectomes, confirming alterations in network topology following its application (Liang et al., 2012; Yan et al., 2013).
The impact of network construction steps on topology

In addition to the aforementioned effects of GSR on network architecture, numerous other steps in the network construction pipeline will substantially affect brain network topology.

For example, the importance of nodes should in principle be determined based on their connectivity; as such, many analyses have used nodes of approximately equal size. However, it may be even more important that regions should be homogeneous entities, respecting anatomical or functional boundaries. It has been suggested, based on simulations, that applying anatomical parcellations to fMRI data can result in distortions of functional network architecture (Smith et al., 2011). This can potentially be offset through the use of smaller regions, within which signal will be more homogeneous (Korhonen et al., 2017). One approach taken by numerous studies to ensure robustness of results is to replicate findings following parcellation of the brain according to several different atlases.

Moreover, while most past studies have used a single group template to define network nodes, attention has recently been shifting towards individualised parcellations based on subject-specific data (Glasser et al., 2016). Given recent reports of subtle differences in functional network architecture between individuals (Braga and Buckner, 2017; Gordon et al., 2017), the application of group templates might be damaging to connectivity estimation. Indeed, a recent study has reported that application of individual data-based parcellations might result in smaller between-subject differences in connection strength, in favour of greater differences in network topography (i.e.: location and spatial arrangement); crucially, these topography-based fingerprints might be more strongly related to behavior and cognition than previously-reported strength-based markers (Kong et al., 2018).

Similarly, the computational process used to define edges will impact network topology. In DWI tractography, long edges are less likely to be detected than short edges, since an increasingly long string of consecutive voxels is decreasingly likely to satisfy tracking criteria; if satisfying them, it is instead increasingly likely to become a false positive connection (Maier-Hein et al., 2017). Similarly, networks estimated using statistical association measures such as the correlation coefficient will present substantially increased transitivity or clustering by construction (Zalesky et al., 2012). This can in theory be avoided through the use of partial measures (such as the partial correlation); however, as discussed above, the application of such measures requires a substantially larger number of samples than regions. Another method for preventing the unintended introduction of non-trivial topological features is to benchmark summary network statistics against appropriate null models (Zalesky et al., 2012; Fornito et al., 2016).
The strongest edges are not necessarily the most likely

Following the definition of nodes, and edges between them, thresholds are commonly applied. However, the decision to retain certain edges and discard others is riddled with several potential (partly related) issues.

One potential issue is the widespread assumption that the strongest edges are also the most likely to be “true”. This is a reasonable assumption, but often precludes the selection of a principled threshold; therefore, networks are commonly thresholded across a range of densities (Fornito et al., 2016). Alternative approaches have been proposed, such as integration across thresholds or “cost-integration” (Ginestet et al., 2011), as well as threshold choice (De Vico Fallani et al., 2017) based on optimisation of the cost-efficiency trade-off (Bullmore and Sporns, 2012). Still, such measures place assumptions on data and, by definition, induce or enhance certain aspects of topological organisation.

Efforts to retain edges based on criteria other than weight have been implemented in the past. It has been suggested that edges in networks based on DWI tractography could be retained based on inter-subject consistency: for example by retaining only edges present in at least 30% of participants (de Reus and van den Heuvel, 2013). Subsequently, it has been suggested that this leads to preferential retention of short edges, which are more likely to be consistent across subjects simply by virtue of being more prominent in tractography-derived networks. Thus, the consistency-based threshold can be applied to subsets of edges binned by length, to better match the empirical distance distribution (Mišić et al., 2015). These approaches, initially developed in the context of sparse connectomes obtained from deterministic tractography, have been generalised to dense connectomes from probabilistic tractography by retaining consistently strong (rather than consistently present) edges (Roberts et al., 2017). The latter method is also potentially applicable to functional connectomes, as derived from fMRI. Still, further work is needed to retain edges most likely to represent "signal" - in both structural and functional networks, and particularly in networks derived using association measures.

Relationship between lower and higher-order properties of the network

Graph theory provides a means of reducing the dimensionality of complex networks, and capturing key features of their architecture through the use of summary statistics. However, it is important to ensure that these measures capture interesting "higher-order" properties of network organisation, and are not trivially dependent on the "lower-order" quantities or processes used to derive them.
For example (as mentioned above), it is known that certain graph theoretical measures show a dependence on network size and density (van Wijk et al., 2010); therefore, networks are generally compared which exhibit the same number of nodes (although there are exceptions to this rule; see Im et al. (2014)) and are thresholded to common density. However, it is known that thresholding to fixed edge density can lead to confounding effects when comparing groups with different distributions of edge weights – in a group with higher edge weights, this would lead to ignoring potentially important edges, while in a group with lower edge weights, this would lead to including weak or “non-significant” edges (van Wijk et al., 2010; Fornito et al., 2012).

**Dimensionality of the network construction pipeline**

Crucially, the network construction pipeline, from raw data to summary measures, involves numerous steps. At each step, multiple decisions can be made, leading to a wide range of possible outcomes (e.g.: Phillips et al., 2015). While some approaches are more popular than others, there is considerable diversity in practice across laboratories. In turn, this is one of the factors responsible for the heterogeneity of findings across the literature.

Thus, studies must be interpreted in context of the methods that have been used in the network construction and analysis process. Specifically, it is not surprising that studies constructing brain networks in different ways (even from the same data) might yield different results, but it is important that in many cases, the interpretation of these results may differ as well. A good example has recently been provided in the context of GSR:

"A simple analogy is analysing the ripples on a pond on a windy day. If the goal is to determine which direction the wind is blowing, one needs to analyse the data with global fluctuations included. If one is trying to determine the location of a small pebble thrown into the pond, regressing out common fluctuations may be critical. Neither analysis is a more accurate representation of the 'true' nature of the pond. Rather, if applied and interpreted correctly, they provide complimentary information." (Murphy and Fox, 2017)

Going forward, it is important that both data (Nichols et al., 2017) and analysis code (Eglen et al., 2017) are made publicly available, so that others may attempt to replicate published findings, and study for themselves the effect of modulating parameters in the network construction and analysis pipeline.
1.4 Thesis structure

This thesis comprises three parts, each of which describes methods for the analysis of brain networks constructed using correlations. These methods differ in their details and specific applicability to structural or functional networks, but all aim to address a common theme: disease-related or developmental shifts in the weight distribution of correlation-derived brain networks have important implications for analysis. Such implications need to be carefully considered to avoid confounding of summary measures of network organisation by the underlying changes in correlations.

Part I focuses on changes in functional correlation networks in patients with schizophrenia, which are known to exhibit shifts in the correlation distribution. Chapter 2 describes a novel probabilistic thresholding procedure, whereby edges are retained in order of decreasing probability (rather than decreasing weight), which is further adjusted for the effects of participant in-scanner motion. Probabilistic thresholding is compared to traditional weight-based thresholding. In Chapter 3, the impact of probabilistic thresholding on network randomisation in schizophrenia is investigated. Network randomisation has previously been reported as a phenotype of schizophrenia, but the possibility has been raised that these findings might have resulted from the application of weight-based, fixed density thresholds to functional networks of patients with shifted correlation distributions.

Subsequently, Parts II and III focus on adolescent development. Abnormal brain maturation during this period has been thought to underlie the emergence of numerous psychiatric disorders (including schizophrenia), but it is still poorly understood. Both structural and functional networks have been reported to undergo changes over adolescence.

Part II focuses on structural covariance networks, constructed using cross-correlations between cortical thickness of pairs of regions across participants. In Chapter 4, novel methods are introduced to quantify changes in structural network architecture, including a sliding-window method to shift from a discrete towards a continuous representation of network development, as well as a bootstrap procedure to estimate consistency of edge-wise associations, which can be used to threshold the network. Developmental results are reported and discussed. In Chapter 5, the robustness of the newly introduced methods and the resulting findings are investigated, including their sensitivity to parameters and properties of the input data.

Part III consists of a single chapter, Chapter 6, which focuses on functional networks constructed using fMRI data from a subset of participants whose structural scans are analysed in Part II. Adolescent development of fully weighted functional networks is studied, focusing first on shifts within the correlation distribution, then on regional maturation and finally
on developmental changes within individual edges. Functional maturation differs between
cortical and subcortical regions, and within cortex is further patterned according to known
systems, with large developmental differences between primary and association cortical
regions.

Finally, findings are summarised in Chapter 7. Their generalisability is discussed, as well
as their limitations - providing suggestions of avenues for future work.
Part I

Probabilistic Thresholding of Functional Networks
CHAPTER 2

PROBABILISTIC THRESHOLDING OF FUNCTIONAL (DYS)CONNECTOMES IN SCHIZOPHRENIA

2.1 Introduction

As topological measures applied to brain networks are non-trivially dependent on the density of the underlying graph (van Wijk et al., 2010), weighted functional connectomes are traditionally thresholded to fixed edge density to enable comparisons of graph-theoretical measures across participants. Analyses using fixed-density thresholding are usually carried out across a range of thresholds to mitigate the fact that the choice of a single fixed density is arbitrary; however, a statistically principled framework for thresholding individual graphs and analysing brain network connectivity has been lacking.

Recently, new wavelet-based methods have been proposed, which enable simultaneous denoising (Patel et al., 2014) and probabilistic inference (Patel and Bullmore, 2016) on functional connectomes constructed from individual subjects. These methods define the spatial variability in effective degrees of freedom (df) at each voxel or region after denoising and motion artefact removal, using wavelet despiking. These local estimates of effective df can then be used to adjust edge-wise P values or probabilities, thus enabling the construction of probabilistically-thresholded graphs. As a result of adjusting edge-wise P values for the motion-related local reductions in df, the strongest edges (displaying the highest values of correlation) are not necessarily the most likely. This is highly relevant in the case of group differences within the correlation distribution: whereas retaining edges in order of decreasing weight completely preserves group shifts in the correlation distribution, these differences are attenuated when retaining edges in order of increasing df-adjusted P value. Beyond the initial presentation of these methods in Patel and Bullmore (2016), their implications for network analysis of functional connectivity have not been fully explored. Here we apply these new methods to resting-state fMRI data of patients with schizophrenia and healthy participants.
Schizophrenia has been described as a disorder involving both disconnection (Friston and Frith, 1995; Friston, 1998) and dysconnectivity (Bullmore et al., 1997; Stephan et al., 2006); the former referring to weaker or missing connections, the latter to aberrant connectivity more generally (Stephan et al., 2006; Fornito et al., 2012). Dysconnectivity in schizophrenia has been extensively studied using multiple methods, including graph theory (see e.g.: Bullmore and Sporns (2009) and Fornito et al. (2013) for reviews on analyses of neuroimaging data using graph theory, and Fornito et al. (2012) and van den Heuvel and Fornito (2014) for its specific applications to schizophrenia). Importantly, patients with schizophrenia have been known to exhibit shifted correlation distributions (Lynall et al., 2010; Zalesky et al., 2012; Lo et al., 2015).

We thresholded functional MRI scans of 56 patients with schizophrenia and 71 healthy controls (the COBRE dataset) based on statistical significance of edges, after taking into account the effects of motion and other artefacts. We aimed to investigate the general implications of probabilistic thresholding on graph theoretic analysis of brain network connectivity. Specific questions included: (i) To what density can each participants’ connectome be reconstructed, while ensuring that all retained edges remain statistically significant after accounting for the effects of motion? Does this density have diagnostic potential? (ii) Conversely, what proportion of connectomes can be reconstructed up to fixed densities generally considered in the literature? (iii) Are probabilistically thresholded connectomes more consistent across participants?

2.2 Methods

2.2.1 MRI data and processing

Raw anatomical and functional MRI scans of 72 patients with schizophrenia and 75 healthy controls were made available by the Mind Research Network and University of New Mexico (http://fcon_1000.projects.nitrc.org/indi/retro/cobre.html). Informed consent was obtained from all subjects according to institutional guidelines required by the Institutional Review Board at the University New Mexico (UNM).

All participants were scanned on a 3 Tesla Siemens TIM scanner. Structural data was collected using a multi-echo MPRAGE (MEMPR) sequence with the following parameters: TR = 2.53 s, TE = [1.64, 3.5, 5.36, 7.22, 9.08] ms, TI = 900 ms, matrix size 256 x 256, 176 slices, voxel size = 1 x 1 x 1 mm³. Resting-state data was collected with echo-planar imaging (EPI), with the following parameters: TR = 2 s, TE = 29 ms, matrix size 64 x 64, 32 slices, voxel size = 3 x 3 x 4 mm³, scan duration = 304 s (152 volumes). Subjects were
instructed to keep their eyes open during the scan. Subject ages ranged from 18 to 65 years old. Diagnostic information was collected using the Structured Clinical Interview used for DSM Disorders (SCID).

Core image processing of the structural and functional images was done in AFNI. This included: skullstrip of the structural image, slice timing correction, rigid-body head movement correction (to the first frame of data), obliquity transform of the functional to the structural image, affine co-registration of the functional to the structural image using a grey matter mask, non-linear standard space transform to the MNI152 template in standard space, spatial smoothing (6mm FWHM) and within-run intensity normalisation to a whole-brain median of 1000. Subsequent time series denoising steps included: voxel-wise wavelet despiking using the BrainWavelet Toolbox (www.brainwavelet.org), segmentation of CSF signal using FSL fast, and linear regression of 6 movement parameters, their first-order derivatives and CSF signal. All pre-processing steps were performed using AFNI software (Cox, 1996), except CSF segmentation which was performed using FSL FAST (Smith et al., 2004), and time series denoising which was done using the BrainWavelet Toolbox for denoising motion artefacts (Patel et al., 2014).

Following data processing, participants with a mean spike percentage (the percentage of gray matter voxels containing a motion-related spike in the wavelet domain, averaged across time points) greater than 7.5% were excluded from further analysis. This threshold was chosen as it was the highest threshold that enabled the elimination of a between-group bias in motion and average effective $df$ (after subject exclusion). This resulted in the exclusion of 15 patients with schizophrenia and 3 healthy controls. Additionally, one patient was excluded due to a truncated run during acquisition, and one healthy control due to reconstruction errors. Thus, 56 patients with schizophrenia and 71 healthy controls were included in the study.

### 2.2.2 Wavelet despiking and estimation of effective degrees of freedom

Wavelet despiking is a method for voxel-wise spatially-adaptive denoising of motion artefacts across frequencies that accounts for the highly non-linear nature of these artefacts. The first step of the algorithm is to perform a maximal overlap discrete wavelet transform (MODWT) on the time series (length = $t$) from each voxel, which derives a set of frequency bands (or scales = $j$) from the signal creating a set of $t \times j$ wavelet coefficients. Denoising is conducted in the time-scale plane on these $t \times j$ coefficients for each voxel. The algorithm identifies coefficients contaminated by large non-stationary phenomena (artifacts) as chains of maximal and minimal wavelet coefficients across frequencies and splits the coefficients for each voxel time series into two additive sets: one representing ‘noise’ coefficients ($\Phi$), the other representing non-noise or ‘signal’ coefficients ($\alpha$), as described in equation 2.1:
\[ \tilde{W}_{X,j,t} = \tilde{W}_{\alpha,j,t} + \tilde{W}_{\Phi,j,t} \]  

(2.1)

where \( \tilde{W}_{X,j,t} \) is the MODWT of voxel time series \( X_t \).

In the final step, the algorithm recomposes the denoised time series from \( \alpha \), setting all \( \Phi = 0 \) (a process known as hard thresholding), to yield a denoised time series of length \( t \).

Effective degrees of freedom (\( \hat{h}_j \) or simply \( df \)) are estimated for each scale in the time-scale plane from the ‘signal’ coefficients, \( \alpha \), using equation 2.2 below, as described in Patel and Bullmore (2016); \( M \) is the number of non-boundary coefficients (please see Patel and Bullmore (2016) for a full description).

\[ \hat{h}_j = \max \left\{ \left\lfloor \frac{M_j - M\Phi,j}{2^j} \right\rfloor, 1 \right\} \]  

(2.2)

This results in estimation of \( \hat{h} \) (\( df \)) for each voxel at each wavelet scale.

2.2.3 Graph construction

Individual networks were constructed using a parcellation of cortex into 470 nodes, created by subparcellating a fusion of the Harvard-Oxford cortical and subcortical atlas and the Oxford thalamic connectivity atlas (Patel and Bullmore, 2016). 50 nodes were excluded due to incomplete coverage between subjects and dropout. Edge weights were calculated as Pearson correlations in the wavelet domain between the remaining 420 nodes, separately for each wavelet scale. Based on previous work indicating that wavelet scale 2 (0.060-0.125 Hz) is the most sensitive to differences between patients with schizophrenia and healthy controls (Lynall et al., 2010), the results primarily focus on this scale; still, the main analyses were repeated at scales 1 and 3 (see table 2.2). For a schematic representation of the graph construction pipeline, see Fig. 2.1A.

Subject-specific \( df \) maps were parcellated using the same template used for parcellating the time series. This gave regional (or nodal) \( df \) estimates for each wavelet scale. When constructing graphs from this set of nodal \( df \), edges were assigned the minimum \( df \) of the connecting nodes. So if node 1 had \( df = 30 \), and node 2 had \( df = 40 \), the edge \( df \) connecting these nodes was assigned \( df = 30 \). The correlation between nodal time series (\( r \)) at each scale (\( j \)) were then converted to two-tailed P values using the Fisher r-to-Z transform and comparing to the standard normal distribution, normalising for edge \( df \):
2.2 Methods

\[ Z_j = 0.5 \cdot \ln \left( \frac{1 + r_j}{1 - r_j} \right) \cdot \sqrt{\hat{n}_j - 3} \]  

(2.3)

Unthresholded functional connectomes were characterised using the global mean correlation and node strength, calculated respectively as the mean of the upper triangular parts and rows (or equally, columns) of each participant’s adjacency matrix. Positive and negative correlations were not separately considered in this analysis.

### 2.2.4 Probabilistic thresholding methods

Fig. 2.1 **Methods of probabilistic graph construction and analysis.** A) Pipeline for graph construction using wavelet \( df \). During pre-processing and denoising with wavelet despiking (Patel et al., 2014), voxel-wise effective degrees of freedom (\( df \)) are extracted (Patel and Bullmore, 2016). Following parcellation of cortex into nodes and construction of a functional correlation network, edge-specific \( df \) are used to obtain edge-specific P values, which (following FDR adjustment for multiple comparisons) can be used to threshold the network. B) Illustration of potential differences between graphs thresholded based on correlations, or \( df \)-adjusted P values. Graph thresholding, either to variable edge density using an absolute threshold (\( \tau \); first column) or to fixed edge density using a proportional threshold (\( \kappa \); second column) has traditionally been performed using correlation (first row). However, application of analogous thresholding methods using \( df \)-adjusted P values (second row) can lead to different topologies.
Throughout this chapter and Chapter 3, the terms “probabilistic thresholding” and “P value thresholding” (or “P-thresholding”) are used interchangeably, as the P value is an estimate of connection probability. However, we note that the method itself is deterministic, in that for a given network and threshold, the same edges will be retained. Moreover, we use the term “significant” to mean FDR-adjusted $P < 0.01$.

An illustration of differences in topological organisation that may arise when thresholding connectomes based on correlations or P values is shown in Fig. 2.1B.

**Fixed P value thresholding**

First, we thresholded each participant’s graph at an FDR-adjusted significance level of $\alpha = 0.01$ – an approach which bears some similarity to (absolute) weight-based thresholding (Fornito et al., 2013) but additionally controls for type I error (“false positives”). We term all edges surviving this probabilistic threshold “significant edges”. Very few negatively-weighted edges survived (within and across participants), and inclusion of these edges had a negligible effect on downstream topological measures. Negative edges were therefore excluded from the main analysis. (Details on the presence of negative edges are presented in Results section 2.3.5.)

To study topological disconnectivity, P-thresholded connectomes for individual subjects were binarised by assigning all retained edges a uniform weight of 1. Upon thresholding and binarisation, many of the individual connectomes fractionated into multiple components. Therefore, we studied the topological disconnectivity within each participant’s connectome as a signal of interest, evaluating it using several global summary measures:

- **Edge density**: the ratio of the number of edges present in the P-thresholded graph to the total number of possible edges.

- **Connected components analysis**: these are sub-graphs within which any node is reachable from any other node by a path. We evaluated both the number of connected components, and the size of the largest component for each participant.

- **The percolation threshold**: this is the threshold that determines connectedness of all nodes. In this case, it is the (FDR-adjusted) P value below which all nodes form part of a single “giant” connected component, while above it the graph begins to fractionate into multiple (dis)connected components.

- Additionally, we calculated the average Euclidean distances spanned by retained edges.

Further, we assessed local measures analogous to the global ones – node degree (the number of edges connected to a node), local edge density (degree normalised by number of
edges in the graph), a nodal connected component score (defined as the size of the component the node is connected to, normalised relative to the largest component), and average distance spanned by a node’s edges.

Summary measures were compared between groups using two-tailed Mann-Whitney U (MWU) non-parametric tests. Effect sizes were quantified using the “simple difference formula” which results in a rank-biserial correlation $r$. All possible combinations of pairs of measurements from the two groups are classified as either favourable ($f$) or unfavourable ($u$) to the null hypothesis; then, the rank-biserial $r$ is calculated as the difference between the two ($r = f - u$; Kerby, 2014). The rank-biserial correlation is a signed value between 1 and -1; we used healthy controls as the reference population, resulting in positive values of $r$ for measures that are increased in schizophrenia, and negative values of $r$ for decreases in schizophrenia.

Finally, we examined whether functional connectomes thresholded using fixed P values exhibit some of the known hallmarks of topological organisation, including the presence of highly connected hub nodes (Sporns et al., 2007) and decomposability into densely intra-connected but sparsely inter-connected modules (Sporns and Betzel, 2016).

### Fixed density thresholding

In a second analysis, we thresholded individual connectomes to fixed edge density (from 1 to 35%, in steps of 1%), adding edges in order of increasing P value. In doing so, we studied the evolution of the maximum $df$-corrected P value per participant as a function of edge density. This analysis also indicates the maximum edge density that one can add edges to by P value for a given participant – once a threshold of $P_{FDR} = 1$ is reached, all remaining edges are equally unlikely and no further edges may be added by P value. Furthermore, we asked what proportion of our participants’ connectomes could be built up to a given fixed edge density, under the condition that all $df$-corrected P values remain statistically significant ($P_{FDR} < 0.01$). In adding edges in order of increasing P value beyond a threshold of $P_{FDR} = 0.01$, an increasing number of negative edges might be added (Fig. 2.10). However, negative edges were again excluded as they had no qualitative impact on the results.

#### 2.2.5 Comparison of probabilistic and correlation-based thresholding

Our final analysis consisted in comparing probabilistic thresholding, where edges are added in order of increasing P value (adjusted for effective $df$), and correlation-based thresholding, where edges are added in order of decreasing correlation. We note that in time series with fixed nominal degrees of freedom (equal to the number of time points, which is an overesti-
mate of the true effective \( df \), the two approaches will lead to identical results, due to the perfectly monotonic relationship between the correlation coefficient and the \( P \) value (Fig. 2.2A). However, following adjustment for effective \( df \), the \( P \) values become \( df \)-dependent – and thus different edges may be added in order of increasing \( P \) and decreasing \( r \) (Fig. 2.2B).

To compare the two approaches explicitly, we constructed for each participant a set of fixed-density graphs (in the range from 1 to 35% edge density) by adding edges in order of decreasing correlation coefficient (\( r \)). We then compared these to networks thresholded in order of increasing \( P \) value (as described above) using several approaches.

We first evaluated the proportion of edges that differ between the two networks, within participants and as a function of edge density.

Further, we examined whether thresholding by \( P \) value results in greater consistency of edges compared to thresholding by correlation. For each thresholding method (\( r \) and \( P \)) and at each edge density, we quantified the consistency of each edge by counting the number of times each particular edge was found across each group (controls and patients). We then converted this data to histograms by counting the number of edges that were observed for each level of consistency. Finally, we assessed the difference in these histograms of consistency between \( r \)- and \( P \)-thresholded networks. The significance of any observed differences was determined by repeating the above procedure 10’000 times, following permutation or \( r \)- and \( P \)-thresholded networks within group; a \( P \) value was calculated as the proportion of permuted differences surpassing the empirical difference. The procedure is illustrated in Fig. 2.8A. Note that we did not consider participants beyond densities at which edges with \( P_{FDR} = 1 \) began to be added (in networks thresholded by \( P \) value).

### 2.2.6 Sensitivity analyses

To ensure robustness of our results, we conducted several ancillary studies.

First, to rule out effects of motion on our results, we examined whether short and long edges were differentially affected by potential residual effects of motion, by examining the relationship between Euclidean distance, and the correlation of edge-wise connectivity and movement (quantified using the frame-wise displacement) across participants (Satterthwaite et al., 2012). We also evaluated group differences between global and regional average \( df \).

Further, we investigated the dependence of our results on the addition of negative edges in \( P \)-thresholded connectomes.

To rule out effects of age on our results, we evaluated within-group correlations between age and the average edge weight (of unthresholded connectomes) and the edge density and number of connected components (of connectomes thresholded by fixed \( P \) value). Furthermore, we investigated differences in these measures between male and female participants.
2.3 Results

2.3.1 Edge weight (correlation) distributions

The inter-regional wavelet correlation coefficients, or edge weights, were normally distributed and predominantly positive in both groups. Mean edge weights were significantly greater in the control group (mean weight: median = 0.37, first and third quartiles ([Q1,Q3]) = [0.29,0.45]) than in the schizophrenia group (mean weight: median = 0.27, [Q1,Q3] = [0.20,0.34]; rank-biserial $r = -0.45$, $P_{MWU} = 1.2 \cdot 10^{-5}$; Fig. 2.3A). Accordingly, the edge weight distribution for the schizophrenia group included more negative correlations than the control distribution.

At a nodal level of analysis, edge weights were significantly reduced in the schizophrenia group compared to the control group at 389 nodes (FDR-adjusted $P_{MWU} < 0.01$; Fig. 2.3B).

Finally, we investigated consistency of results across wavelet scales 1 (0.125-0.25 Hz) and 3 (0.03-0.06 Hz), as well as across more lenient and stringent FDR-adjusted P value thresholds of $\alpha = 0.05$ and $\alpha = 0.001$. At wavelet scale 4 (0.015-0.03 Hz), there were insufficient degrees of freedom for the addition of even a single edge.
Fig. 2.3 **Decreased (unthresholded) correlations in patients with schizophrenia.** A) Edge weights in unthresholded networks, as distributions within individual participants (thin lines) as well as averaged within groups (bold lines; top), and averaged within participants (bottom). Distributions were constructed using kernel density estimates. B) Maps of nodal connectivity strength, calculated as average correlation per node. (i) Median node strength of healthy controls, (ii) median node strength of patients with schizophrenia and (iii) difference in median node strength between groups (controls – patients). In map (iii), only the 389 nodes showing a significant difference are visualised.

There were no significant differences in nodal connectivity strength attributable to greater connectivity strength in participants with schizophrenia compared to healthy controls.

### 2.3.2 Properties of probabilistically-thresholded connectomes

Functional connectomes of patients with schizophrenia (thresholded at $P_{FDR} < 0.01$) were significantly more disconnected than those of healthy controls, exhibiting a lower edge density (rank-biserial $r = -0.47$, $P_{MWU} = 6.5 \cdot 10^{-6}$; Fig. 2.4Ai), higher numbers of connected components (rank-biserial $r = 0.38$, $P_{MWU} = 2.1 \cdot 10^{-4}$; Fig. 2.4Aii), and consequently a higher percolation threshold (rank-biserial $r = 0.20$, $P_{MWU} = 0.052$). In patients with schizophrenia, the median connection density of these probabilistically-thresholded graphs was 4.0%, $[Q1,Q3] = [2.6%, 7.9%]$; the median number of connected components was 17, $[Q1,Q3] = [9, 30]$; and the median percolation threshold $P$ value was 0.57, $[Q1,Q3] = [0.16, 1]$. In the equivalently thresholded graphs for the control group, the median connection density was 13.8%, $[Q1,Q3] = [4.7%, 28.4%]$; the median number of connected components was 8,
2.3 Results

Fig. 2.4 Connectomes thresholded by fixed P value are more disconnected in patients with schizophrenia. A) Differences in i) edge density and ii) the number of connected components, with P values corresponding to two-tailed Mann-Whitney U tests. The x-axes indicate histogram frequency normalised by the number of participants (in each group). B) Illustrations of reduced edge density in schizophrenia, depicted as edges present in at least 90% participants per group, using brain network plots (only nodes connected to an edge are visualised).

\[ [Q1,Q3] = [3.25,17.75] \text{; and the median percolation threshold P value was 0.27, } [Q1,Q3] = [0.069,0.82]. \]

The reduced edge density of connectomes of patients with schizophrenia is seen in a visualisation of significant edges present in at least 90% of participants in each group (Fig. 2.4B).

Edges in the probabilistically-thresholded networks spanned shorter distances in patients with schizophrenia (rank-biserial \( r = -0.45, P_{MWU} = 1.3 \cdot 10^{-5} \)). The median average connection distance in patients was 48.7 mm, \([Q1,Q3] = [40.5 \text{ mm}, 58.0 \text{ mm}] \), while the median average connection distance in controls was 60.6 mm, \([Q1,Q3] = [52.6 \text{ mm}, 69.2 \text{ mm}] \).

Finally, the variable-density networks presented the same hallmarks of topological organisation as fixed-density networks. Both groups exhibited spatially heterogeneous degree distributions, with highly connected “hub” nodes located in parietal and occipital cortices (consistent with other studies that did not apply global signal regression, which tends to shift hubs from primary to association areas; Yan et al. (2013)). Similarly to (unthresholded) node strength, patients with schizophrenia exhibited decreased node degree (FDR-adjusted \( P_{MWU} < 0.01 \)) at 382/420 nodes (Fig. 2.5A). However, these decreases were driven by group differences in edge density – when local connectivity was assessed using a measure of local edge density (degree normalised by the participants’ edge density) no nodes showed significant differences between the two groups. Similarly, the two groups showed no significant
Fig. 2.5 Nodal degree and modular organisation of P value thresholded networks. A) Maps of median nodal degree, for (i) healthy controls and (ii) patients with schizophrenia, as well as (iii) group differences between the two (only the 382 nodes showing significant differences between groups are visualised). B) Modular organisation of P value thresholded networks, obtained as a within-group consensus across 100 runs of the Louvain community algorithm (with resolution parameter $\gamma = 1$) on each participant’s connectome, for (i) healthy controls, and (ii) patients with schizophrenia.

differences in the size of the connected component per node (normalised by the largest component in the graph). Furthermore, the P-thresholded graphs were decomposable into densely intra-connected but sparsely inter-connected modules using the Louvain community algorithm (Blondel et al., 2008). For a resolution parameter of $\gamma = 1$, a consensus modular organisation (Lancichinetti and Fortunato, 2012) across participants (100 runs per participant) within each group yielded four modules per group (Fig. 2.5B), which were highly similar – only 26/420 nodes were assigned to different modules in the two groups.

2.3.3 To what density can functional connectomes be built?

Next, we used probabilistic thresholding to address an important question: to what density can we build functional connectomes to avoid adding false-positive edges? Here, we built networks by adding edges in order of increasing $P_{\text{FDR}}$ and thresholded to a fixed edge density (see Methods). We found that the largest edge $P_{\text{FDR}}$ in any given subject’s network rose rapidly as the network density was increased from 1 to 35% (Fig. 2.6A). In other words, many of the graphs needed to be constrained at very low connection densities to prevent
2.3 Results

Fig. 2.6 Thresholding connectomes to fixed edge density (based on P values). A) Maximal FDR-adjusted P values as a function of fixed edge density threshold, for individual participants (thin lines) and averaged within groups (bold lines). B) Number of participants whose edges satisfy $P_{FDR} < 0.01$ (continuous lines with markers) and $P_{FDR} < 1$ (dotted lines), as a function of edge density. Once a P value of $P_{FDR} = 1$ is reached, further edges cannot be added by P value as all remaining edges are equally unlikely.

inclusion of non-significant edges; this was true for both groups. Accordingly, if we require all edges in any given subject’s network to have $P_{FDR} < 0.01$ (which we term “significant edges”), the number of subjects meeting this criterion drops off rapidly as edge density is increased. The drop-off is faster for patients with schizophrenia (Fig. 2.6B) meaning that the maximum connection density that graphs can be built to, while ensuring that all participants’ connectomes contain only significant edges (edges with $P_{FDR} < 0.01$), is much lower in the schizophrenia group. For example, the maximum edge density while ensuring that 95% of connectomes contain only significant edges is only 2% in the control group and 1% in the schizophrenia group. Similarly, the maximum edge density while ensuring that 50% of connectomes contain only significant edges is 13% in the control group and 4% in the schizophrenia group.

2.3.4 Comparing r and P based thresholding

When constructing correlations using an equal number of data points (i.e.: time points in fMRI time series), and assuming that $N(df) = N(\text{time-points})$, the relationship between the Pearson correlation coefficient r and the corresponding P value is perfectly monotonic (Fig. 2.2A). In this case, adding edges to a graph in order of (i) decreasing r or (ii) increasing P leads to identical topologies. However, wavelet-despiked time series exhibit regional heterogeneity in effective $df$ after denoising for motion, which means that the relationship between r and P isn’t perfectly monotonic anymore (Fig. 2.2B). In this case, different edges may be added in order of (i) decreasing r or (ii) increasing P.
Fig. 2.7 Proportion of edges that differ between \( r \)- and \( P \)-thresholding. The percentage of edges that differ between functional connectomes thresholded by increasing \( P \) or decreasing \( r \), as a function of fixed edge density, within healthy controls (left) and patients with schizophrenia (right). At low edge densities, \( \sim 2.8\% \) edges differ between the two approaches, while at higher edge densities, this decreases to \( \sim 1.2\% \) differing edges.

We quantified the proportion of edges that differ between \( r \)- and \( P \)-thresholded connectomes, as a function of edge density (Fig. 2.7). The difference lay between 0 and 10\% of edges present at a given edge density, with an average of \( \sim 2.8\% \) edges differing between \( r \) and \( P \)-thresholded connectomes at low edge densities, slowly decreasing to an average of \( \sim 1.2\% \) edges differing at higher edge densities. As with previous analyses involving thresholding of connectomes to fixed edge density by increasing \( P \) value, we only included participants with all edge \( P_{\text{FDR}} < 1 \) at each density.

Fig. 2.8 (Figure on the following page.) A) At each edge density and for each thresholding method (\( r \) and \( P \)), we quantified the consistency of each edge by counting the number of times each edge was present across the group at a given density. We then counted the number of edges that were found to occur at each level of consistency (i.e.: the frequency of edges as a function of consistency). We did so for both \( r \)- and \( P \)-thresholding methods, and then evaluated the difference between the two. We estimated the significance of these differences by repeating the above procedure 10’000 times following permutation or \( r \)- and \( P \)-thresholded networks within group; \( P \) values were estimated as the proportions of empirical differences surpassing the permuted differences. As in previous analyses where networks were thresholded by fixed \( P \) value, we only considered participants with edge \( P_{\text{FDR}} < 1 \) at each density. B) Connectomes thresholded by decreasing \( P \) value showed an overall increased consistency of edges compared to connectomes thresholded by decreasing \( r \). This was evidenced by an increased frequency of edges absent in all participants in \( P \)-thresholded networks (left inset histograms), in both (i) healthy controls and (ii) patients with schizophrenia.
Fig. 2.8 P-thresholded connectomes show increased edge consistency compared to r-thresholded connectomes. (Continued on the previous page.)
Consistency of edges in r- vs. P-thresholded connectomes

We further compared the consistency with which edges were present within both groups when thresholding in order of decreasing correlation or increasing P value (Fig. 2.8A). As in previous analyses where networks were thresholded by fixed P value, we excluded participants whose connectomes contained edges with $P_{\text{FDR}} = 1$. Exclusion of these subjects is indicated by a drop-off in the maximum possible consistency at higher densities (grey panels in top-right areas of heatmaps in Fig. 2.8B).

We found a generally increased edge consistency in P-thresholded connectomes relative to r-thresholded ones, in both groups. The general pattern is of fewer edges being present in lower numbers of participants (blue bands on the left hand-side of Fig. 2.8B i and ii), and of more edges being present in a greater number of participants (a prevalence of light red on the right hand side of Fig. 2.8B i and ii); this is particularly evident at higher connection densities. However, while these differences were generally not significant (except at the lowest edge densities), there was an overall increase of consistency in P-thresholded networks compared to r-thresholded networks, as evidenced by an increased frequency of edges absent from all participants when thresholding by P relative to r (left inset in Fig. 2.8B i and ii). This difference was significant at numerous edge densities, both within healthy controls ($P_{\text{perm}} < 0.01$ at 11-12%, 14-24%, 26-28%, 30% and 35%; Fig. 2.8Bi) and within patients with schizophrenia ($P_{\text{perm}} < 0.01$ at 9% and 13-35%; Fig. 2.8Bii).

2.3.5 Sensitivity analyses

(In)dependence of results on motion

To confirm that the wavelet despiking algorithm had satisfactorily corrected the fMRI time series for effects of head micro-movements during scanning, we demonstrated that correlations between pairs of regional fMRI time series showed no distance-dependent artefacts (Satterthwaite et al., 2012; Fig. 2.9A).

We also found that there was no significant difference between groups in the global mean effective $df$ (rank bi-serial $r = -0.16$, $P_{\text{MWU}} = 0.12$; Fig. 2.9B). The median of the mean effective $df$ across patients with schizophrenia was 36.4, [Q1,Q3] = [35.7,36.7], while the median of the mean effective $df$ across healthy controls was 36.6, [Q1,Q3] = [36.2,36.7]. Moreover, there were no significant between group differences in nodal effective $df$ (Fig. 2.9D; all FDR-adjusted nodal $P_{\text{MWU}} > 0.12$). These results indicate that the severity of head movement artefact, and therefore the number of $df$ lost to wavelet despiking, was not significantly different between healthy controls and patients with schizophrenia.
Fig. 2.9 Independence of results on motion. A) The correlation between functional connectivity (evaluated as the correlation between denoised regional BOLD time series) shows no dependence on the Euclidean distance between regional centroids, demonstrating adequate denoising. B) The (global) average degrees of freedom ($df$) per participant show no difference between groups. C) Although node degree in networks thresholded at fixed P value correlates with nodal $df$ within participants, there is no significant difference between groups in the magnitude of these relationships. In panels B and C, the x-axis indicates histogram frequency normalised by the number of participants (in each group). D) Locally, the regional $df$ (visualised as median $df$ across participants) shows no significant difference between (i) healthy controls and (ii) patients with schizophrenia.
Fig. 2.10 **Presence of negative edges in networks thresholded in order of increasing P-value.** A) In networks thresholded at fixed P-value ($P_{FDR} < 0.01$), there is no clear relationship between the number of (significant) positively and negatively weighted edges connected to a node (only nodes with at least one negative edge were considered). B) In networks thresholded to variable edge density in order of increasing P-value, (i) a larger proportion of (mostly non-significant) negative edges may be added, especially at higher densities. (ii) However, the number of participants exhibiting large proportions of negative edges remains low.

Although node degree is correlated to regional $df$ within participants (median [Q1,Q3] Spearman $\rho = 0.23$ [0.11,0.34] for healthy controls, 0.21 [0.08,0.36] for patients with schizophrenia), as already reported for node strength in Patel and Bullmore (2016), there is no difference in the extent of these relationships between the two groups (rank-biserial $r = -0.036$, $P = 0.73$; Fig. 2.9C).

Finally, the mean spike percentage, a measure of the extent of denoising per participant, does not correlate with the average (unthresholded) correlation, within healthy controls ($\rho = 0.03$, $P = 0.83$) or within patients with schizophrenia ($\rho = 0.15$, $P = 0.27$); nor with the edge density of functional connectomes thresholded at fixed P value, within healthy controls ($\rho = 0.15$, $P = 0.21$) or within patients with schizophrenia ($\rho = -0.03$, $P = 0.81$).

**(In)dependence of results on addition of negative edges**

Upon thresholding at fixed P-values (FDR-adjusted significance level of $\alpha = 0.01$), only a small proportion of connectomes exhibited negative edges (negative correlations) – 5 healthy controls (7.0%) and 6 patients with schizophrenia (10.7%), mostly in small numbers (the
maximum number of negative edges in a healthy network was 22, corresponding to 0.025% of edges in the network, while the maximum number in a patient’s network was 116, corresponding to 0.13% edges). Due to their low numbers, adding these edges to the binarised connectomes has almost no effect on the edge densities or numbers of connected components. Furthermore, although the negative edges tend to be spatially clustered within each connectome which contains them, they present no overlap across participants, and they are not preferentially located at nodes with many significant positive correlations (correlation between positive and negative degree for nodes with negative edges, across groups and participants: $\rho = 0.098, P = 0.26$; Fig. 2.10A).

When thresholding connectomes to fixed density in order of increasing P-values, a larger proportion of (mostly non-significant) negative edges may be added, particularly at higher edge densities (Fig. 2.10Bi). However, the proportion of participants exhibiting large numbers of negative edges remains relatively low (Fig. 2.10Bii). Moreover, the addition of these negative edges has no qualitative effect on results presented herein.

The occurrence of negative edges in functional connectomes and their origin (whether physiological or artefactual), is an outstanding question within the neuroimaging community (Schwarz and McGonigle, 2011).

(In)dependence of results on covariates and parameters

We evaluated within-group correlations of three connectome measures with age: the average edge weight (of unthresholded connectomes), as well as the edge density and the number of (dis)connected components (of P value thresholded connectomes). Only one measure presented a significant correlation – the number of connected components, within patients with schizophrenia (but not within healthy controls; Table 2.1). As only one measure (of three tested) presented an effect of age, within only one group, we conclude that age had no substantial effect on our group differences. Furthermore, we evaluated differences in the same measures between genders, none of which were significant, in either group (Table 2.1).

Finally, we confirmed that our results were qualitatively consistent across wavelet scales 1 (0.125-0.25 Hz) and 3 (0.03-0.06 Hz), as well across more lenient and stringent FDR-adjusted P value thresholds of $\alpha = 0.05$ and $\alpha = 0.001$. For results of these analyses (including median values and interquartile ranges per group, as well as effect sizes and P values for group differences), see Tables 2.2 and 2.3.
Table 2.1 (In)dependence of main results on age and gender. Measures inspected for effects of age and gender (within groups) were the average edge weight (of unthresholded connectomes) as well as the edge density and number of connected components (of P-value thresholded connectomes). Effects of age were evaluated using Spearman’s \( \rho \) correlations, and are indicated as \( \rho (P) \). Effects of gender were evaluated using the two-tailed Mann-Whitney U test between groups, and are reported as \( r (P_{MWU}) \), where \( r \) refers to the rank-biserial correlation.

<table>
<thead>
<tr>
<th>Variable / test used</th>
<th>Age / Spearman’s ( \rho ) (P)</th>
<th>Gender / rank bi-serial ( r ) (( P_{MWU} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measure</td>
<td>Ctrl</td>
<td>Schz</td>
</tr>
<tr>
<td>( \mu ) correlation (unthr.)</td>
<td>-0.12 (0.32)</td>
<td>-0.24 (0.08)</td>
</tr>
<tr>
<td>edge density (P-thr.)</td>
<td>-0.09 (0.46)</td>
<td>-0.17 (0.20)</td>
</tr>
<tr>
<td># conn. comp. (P-thr.)</td>
<td>0.13 (0.29)</td>
<td>0.33 (0.014)</td>
</tr>
</tbody>
</table>

Table 2.2 Consistency of results across wavelet scales. Group differences in the main measures were investigated at wavelet scales 1 (0.125-0.25 Hz), 2 (0.06-0.125 Hz) and 3 (0.03-0.06 Hz). In all cases, connectomes were thresholded at an FDR-adjusted threshold of \( \alpha = 0.01 \). Measures inspected for consistency across scales were the average edge weight (of unthresholded connectomes) as well as the edge density and number of connected components (of P-value thresholded connectomes, thresholded at \( P_{FDR} < 0.01 \)). Between-group differences were evaluated using the two-tailed Mann-Whitney U test, and are reported as \( r (P_{MWU}) \), where \( r \) refers to the rank-biserial correlation. We note that at wavelet scale 4 (0.015-0.03 Hz), connectomes could not be constructed as no edges satisfied \( P_{FDR} < 0.01 \).
2.4 Discussion

In this chapter we demonstrate novel applications for probabilistically-thresholded functional connectomes based on edge-specific P values adjusted for loss of degrees of freedom (df) due to motion (Patel and Bullmore, 2016). We apply these methods to a population of healthy controls and patients with schizophrenia, and demonstrate reduced edge density and increased disconnectedness in patient connectomes when thresholding to variable density by P value (at P_{FDR} < 0.01). We further demonstrate that only few individual connectomes can be constructed to the typically studied densities of 5-30%. Routinely thresholding to these densities without accounting for edge probabilities increases the risk of type I error. Conversely, using the wavelet-despiking-based probabilistic connectivity methods we describe allows identification of the maximum connection density to which a connectome can be built to enable control over false positive results. Finally, we explicitly compare the application of thresholding connectomes by P value (following adjustment for effective df) to existing thresholding methods based on correlation (equivalent to assuming nominal df), demonstrating that different edges are retained, and that the edges retained based on increasing P value are more consistent across subjects than edges retained based on decreasing correlation.

<table>
<thead>
<tr>
<th>α_{FDR}</th>
<th>Measure</th>
<th>Ctrl</th>
<th>Schz</th>
<th>rank-biserial r (P_{MWU})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>edge density</td>
<td>23.9% [9.1%,44.4%]</td>
<td>7.5% [4.9%,15.9%]</td>
<td>-0.47 (6.5·10^{-6})</td>
</tr>
<tr>
<td></td>
<td># conn. comp.</td>
<td>3 [2.8]</td>
<td>7 [3.5,12]</td>
<td>0.30 (0.0036)</td>
</tr>
<tr>
<td>0.01</td>
<td>edge density</td>
<td>13.8% [4.7%,28.4%]</td>
<td>4.0% [2.6%,7.9%]</td>
<td>-0.47 (6.5·10^{-6})</td>
</tr>
<tr>
<td></td>
<td># conn. comp.</td>
<td>8 [3.25,17.75]</td>
<td>17 [9,30]</td>
<td>0.38 (2.1·10^{-4})</td>
</tr>
<tr>
<td>0.001</td>
<td>edge density</td>
<td>6.8% [2.1%,13.6%]</td>
<td>1.8% [1.2%,3.5%]</td>
<td>-0.47 (6.1·10^{-6})</td>
</tr>
<tr>
<td></td>
<td># conn. comp.</td>
<td>21 [8.25,39.5]</td>
<td>51 [26.5,77.5]</td>
<td>0.44 (2.5·10^{-5})</td>
</tr>
</tbody>
</table>

Table 2.3 Consistency of results across thresholds of edge significance. Group differences in the main measures were investigated at FDR-adjusted thresholds of α_{FDR} = {0.05,0.01,0.001}. All results correspond to wavelet scale 2 (0.06-0.125 Hz). Measures inspected for consistency across thresholds were the edge density and number of connected components. Between-group differences were evaluated using the two-tailed Mann-Whitney U test, and are reported as r (P), where r refers to the rank-biserial correlation.
2.4.1 Thresholding methods

We note that there may have been previous studies of functional connectomes where association matrices were thresholded based on P values; however, these have assumed nominal df (number of points in the time series), which we know to be an over-estimate of the effective df (Patel and Bullmore, 2016). Additionally, in a graph where all nodes possess equal df, the relationship between the Pearson correlation r (the most common measure of association between regional neurophysiological time series) and the corresponding P value is perfectly monotonic (that is: as r increases, P decreases, and a given value of r maps to a single value of P). In such a network, thresholding by decreasing correlation or by increasing P value will lead to identical topologies. For this reason, application of the probabilistic thresholding methods described herein requires a spatially heterogeneous map of effective degrees of freedom of the neurophysiological time-series, which are lower than the nominally assumed "N(df)=N(time-points)" and which are subject-specific. To the best of our knowledge, wavelet despiking (Patel and Bullmore, 2016) is the only currently available method to provide such a map on the basis of spatially heterogeneous effects of participant in-scanner motion. Wavelet despiking takes into account the inhomogeneity of artefacts in space and time, enabling the construction of probabilistic connectomes after quantifying the spatially and temporally inhomogeneous loss of df. This is what gives rise to differences in topological organisation between functional connectomes thresholded (to fixed density) based on correlations or df-corrected P values. Although only small proportions of edges differ between connectomes thresholded by increasing df-adjusted P value or decreasing correlation (up to ~10%, depending on the participant and edge density), the probabilistically-thresholded networks show increased consistency across subjects, consistent with the exclusion of “noisier” edges at any given edge density.

Beyond hard thresholding to arbitrary densities, global probabilistic methods have been proposed which estimate dependence of the minimum absolute threshold on the number of samples (fMRI frames) and the number of nodes, whilst maintaining appropriate global FWER control (De Vico Fallani et al., 2014). Other methods include integrating across a range of arbitrary thresholds (Ginestet et al., 2011), or attempting to choose thresholds in an informed, non-arbitrary manner (De Vico Fallani et al., 2017) based on the cost-efficiency trade-off (Bullmore and Sporns, 2012). Methods presented herein extend beyond global probabilistic and principled thresholding methods by taking into account the spatially and temporally inhomogenous loss of df and thus the heterogeneity of correlation probabilities across the brain.

It is worth stating that thresholding is a difficult problem. While it seems sensible to eliminate non-significant estimates of functional associations between regions, very tight
control over type I error (false positive rate) and thus thresholding to very sparse densities may theoretically lead to increased type II error (false negative rate) and removal of signal. Indeed, weak correlations may contain diagnostic information, specifically with respect to schizophrenia (Bassett et al., 2012). Thus, “soft thresholding” methods designed to suppress rather than remove weaker connections (Schwarz and McGonigle, 2011), and methods to analyse unthresholded (fully connected) weighted connectomes (Rubinov and Sporns, 2011) have much value. Moreover, while the impact of the trade-off between sensitivity and specificity on topological measures of network organisation has recently been explored in the context of DWI data (Zalesky et al., 2016), further exploration of this trade-off in the context of fMRI analysis (or correlation-derived brain networks more generally) is warranted.

2.4.2 Further considerations

We note that the methods of adjusting regional $df$ and consequently edge-wise P values for the effects of spatially (and temporally) heterogeneous denoising using wavelet despiking, proposed in Patel and Bullmore (2016) and applied here, do not provide a universal solution to dealing with all noisy data. The $df$ correction is needed to remove bias in interpretability of connectivity values; that is, to disentangle whether low connectivity is due to low $df$ or due to low intrinsic connectivity, and subsequently to control for type I error. While application of wavelet despiking will denoise regional time series and subsequent adjustment of P values for effective $df$ will take the effects of denoising into account when thresholding, these methods (like all other methods) are unable to “restore” the lost signal in these regions. Thus, users of the methods presented herein should remain aware of potential biases, such as (i) group differences in average or regional $df$ (not present in the current analyses), (ii) within-subject relationships between regional $df$ and connectivity (present in the current analyses, but not different between groups) or (iii) exclusion of a different number of subjects from each group (whether due to high motion at the beginning of the analysis pipeline, or low edge probability when thresholding to high edge densities), leading to potential imbalances in sample size between groups. Taken together, the methods applied herein enable mitigation of the effects of motion on functional connectomes; however, they do not offer a panacea to attempted analysis of low quality data – whether due to high motion, low signal-to-noise ratio or other reasons.

Furthermore, the ability to robustly estimate associations between the activity of pairs of regions depends on factors other than motion. Perhaps the most important of these is the length of the time series. Earlier reports suggested that estimates of functional correlation strength stabilise within five minutes (Van Dijk et al., 2010) and that graph-theoretical measures of functional connectome organisation stabilise after only two minutes (Whitlow...
et al., 2011). However, more recent studies suggest that reliability does substantially increase with increasing scan duration, plateauing at 12 minutes (Birn et al., 2013), with accordingly increased reliability of 14-minute scans relative to 7 minute-scans (Termenon et al., 2016). A recent meta-analysis confirmed increasing test-retest reliability of graph-theoretic fMRI studies with increasing scan duration, especially beyond five minutes (Andellini et al., 2015). Interestingly, whole brain reliability seemed stable with increasing sampling rate for scans of equal duration (Liao et al., 2013), suggesting that the duration of the acquisition has importance above and beyond the sampling rate. From a purely statistical point of view, the larger the number of samples (either longer time series or faster sampling rate), the larger the number of effective $df$; this should translate to a higher density a graph can be built to while ensuring control over type I error.

We also note that the dependence of network topology on factors such as scan duration or (in the case of P value thresholding) the significance level (e.g.: $\alpha = 0.05, 0.01$ or 0.001) precludes us from claiming that there exists a single “true” functional network topology that our method is able to recover – rather, the topology will be conditional on such factors. Still, we believe that in limiting the inclusion of spurious edges, our method comes closer than existing thresholding approaches to estimating the accurately denoised topology.

2.4.3 Future work

First, loss of power might arise from the averaging of voxel-wise signals over functionally inhomogeneous regions of interest. Random sub-parcellations of anatomical atlases (Cammoun et al., 2012; Romero-Garcia et al., 2012), as used here, lead to hundreds of regions whose small size should limit the spatial blurring of functional boundaries. Data-driven parcellations might improve the ability to detect changes in functional connectivity across conditions and development (Yeo et al., 2011; Glasser et al., 2016).

Furthermore, new multi-variate methods of inter-regional association are being developed which appear more accurate than simple correlations (Geerligs et al., 2016); extending such methods to take into account the regionally heterogeneous loss of degrees of freedom due to motion and other scanning artifacts (Patel and Bullmore, 2016) should yield ever-more precise estimates of functional connectivity.

Analysis of dynamic variability of functional connectivity is an increasingly popular avenue (e.g.: Zalesky et al., 2014; Karahanoğlu and Van De Ville, 2015) that we did not explore here. In this context, the importance of denoising methods that preserve the structure of neurophysiological time series (e.g.: Kundu et al., 2012; Patel et al., 2014), and can further adjust estimates of the optimal sliding-window size (Leonardi and Van De Ville, 2015; Zalesky and Breakspear, 2015) to ensure fixed effective $df$ across time (Patel and Bullmore,
should become increasingly clear. Methods such as frame censoring (scrubbing; Power et al., 2012), widely used in the neuroimaging community, are unsuited to denoise artefacts in such cases as they disrupt the temporal structure of the BOLD signal.

Beyond altered connectivity, intrinsic properties of regional neurophysiological signal are altered in schizophrenia. Such alterations are found in the power (Zalesky et al., 2012; Yang et al., 2014) and entropy (Yang et al., 2015) of the BOLD signal. Interestingly, these alterations correlate to measures of functional dysconnectivity in patients with schizophrenia, although regional and inter-regional signal properties may be differentially sensitive to disease deficits (Bassett et al., 2012; Zalesky et al., 2012).

Finally, the lack of clinical or behavioural measures prevented us from evaluating the true diagnostic relevance of the probabilistic thresholding methods presented here. Studying such relationships is a necessary target of future work, if fMRI connectomics are to become clinically useful (Matthews and Hampshire, 2016). An important question is whether probabilistically thresholded functional connectomes lead to stronger relationships between measures of graph (dys)connectivity and cognitive, behavioural or clinical scores. Still, given the likely multivariate nature of relationships between brain connectivity and cognition (Mišić and Sporns, 2016), identifying such relationships is non-trivial.
CHAPTER 3

EFFECTS OF PROBABILISTIC THRESHOLDING
ON NETWORK RANDOMISATION IN SCHIZOPHRENIA

3.1 Introduction

Having presented, in Chapter 2, a novel method for probabilistically thresholding correlation-derived functional connectomes, we wished to investigate its implications on "higher-order" graph theoretical measures calculated using connectomes thresholded to fixed edge density. We retain our focus on schizophrenia, which has been extensively studied using graph theory - particularly using sparse, fixed-density connectomes.

Graph-theoretical studies of brain networks in schizophrenia have generally reported global reductions in both structural (eg: van den Heuvel et al., 2010; Zalesky et al., 2011; Griffa et al., 2015) and functional (Lynall et al., 2010; Zalesky et al., 2012; Lo et al., 2015) connectivity in this disease. Some functional studies have shown localised increases in functional connectivity (Liu et al., 2008; Skudlarski et al., 2010), although these may reflect differences in pre-processing (e.g.: use of partial correlations (Liu et al., 2008) or zero-centering of the correlation distributions (Skudlarski et al., 2010)). Furthermore, functional connectivity studies generally agree that brain networks are topologically altered in schizophrenia, although the nature of these changes and the specific topological measures used to assess them vary between studies (for review, see Fornito et al. (2012) and van den Heuvel and Fornito (2014)).

The popularity of fixed-density thresholding is likely due to the known dependence of traditional higher-order graph-theoretical measures on edge density (van Wijk et al., 2010). Several studies converge on topological alterations such as increased efficiency and/or reduced clustering, consistent with a subtle randomisation of the connectome in schizophrenia (Rubinov et al., 2009; Alexander-Bloch et al., 2010; Lynall et al., 2010); this has been proposed as an endophenotype of the disorder (Lo et al., 2015). However, it has been
hypothesized that this “randomisation” might result from the application of fixed-density thresholds to functional connectomes of patients with schizophrenia, presenting decreased edge weights:

“...in the presence of a global reduction of mean functional connectivity in patients [...] any analysis of graphs matched for connection density, \( k \), will result in the inclusion of proportionally more low-value (non-significant) edges in patients’ networks. If these values merely reflect noise, their inclusion will produce a more random topology.” (Fornito et al., 2012).

A recent study demonstrated that group differences in mean functional connectivity (correlation) do indeed lead to group differences in topological organisation, and proposed to correct for between-group differences in mean connectivity using regression or permutation (van den Heuvel et al., 2017).

In this context we used data from patients with schizophrenia to revisit the topological randomisation hypothesis, using the new statistically principled thresholding methods presented in Chapter 2. Specifically, we wished to find out to what extent differences in topological organization between healthy controls and patients with schizophrenia are sensitive to (or driven by) “non-significant” edges usually added when thresholding connectomes to fixed density based on correlation.

### 3.2 Methods

#### 3.2.1 MRI data and processing

Data used for analysis were identical as in Chapter 2. For full details on data acquisition and processing, see Chapter 2; main details are briefly summarised below. Data consist of MRI scans of 72 patients with schizophrenia and 75 healthy controls, made available by the Mind Research Network and University of New Mexico. Participants were scanned on a 3 Tesla Siemens TIM scanner, using both an anatomical sequence (voxel size = 1 x 1 x 1 mm\(^3\)) and functional resting-state sequence (TR = 2 s, voxel size = 3 x 3 x 4 mm\(^3\)).

Images were processed using AFNI (Cox, 1996) and the BrainWavelet Toolbox for denoising motion artefacts (Patel et al., 2014) and estimation of effective \( df \). Participants were excluded if they presented excessive motion or reconstruction errors, resulting in inclusion of 56 patients with schizophrenia and 71 healthy controls in the study.

Individual networks were constructed using 420 cortical nodes (Patel and Bullmore, 2016), retained after exclusion of 50 nodes from a 470-node parcellation (due to incomplete coverage
between subjects and dropout). Edge weights were calculated as Pearson correlations between nodes in the wavelet domain, at wavelet scale 2 (0.060-0.125 Hz). Subject-specific $df$ maps were parcellated using the same cortical template. When constructing graphs, edges were assigned the minimum $df$ of the connecting nodes. The correlation values between nodal time series were then converted to two-tailed P values using the Fisher r-to-Z transform, normalising for edge $df$.

### 3.2.2 Topology in networks probabilistically thresholded to fixed density

We thresholded individual connectomes to fixed edge density (from 1 to 35%, in steps of 1%), adding edges in order of increasing P value (as described in Chapter 2).

For each individual’s fixed-density graph, we assessed topological integration and segregation. Global integration was quantified using the global efficiency, which is defined as the average of the inverse shortest path length (Latora and Marchiori, 2001). Global segregation was quantified using transitivity, which is the ratio between numbers of triangles and connected triples of nodes in the network (Newman, 2010). We note that the interpretation of transitivity as a measure of topological segregation relies on its application to sparse networks (here, 1-35% edge density); transitivity is maximal in the extreme case of a fully connected graph, in which one might also say that integration is maximal. We did not enforce node-connectedness (e.g.: with minimum spanning tree (MST) thresholding (Alexander-Bloch et al., 2010; Fornito et al., 2016)), as doing so would introduce non-significant edges. However, we quantified the number of connected components in the fixed-density networks, for each participant and at each edge density.

Using these measures, we investigated whether topological randomisation in schizophrenia reported in the literature may be driven by inclusion of non-significant edges (Fornito et al., 2012), which we define as any edge with $P_{FDR} \geq 0.01$. This analysis was conducted in three parts:

1. We first compared the topology between groups at fixed connection densities, adding edges to individual connectomes by increasing P value (until we reached $P_{FDR} = 1$), but disregarding whether we had included non-significant edges. This was our comparator to the literature where connectomes are commonly thresholded to fixed density regardless of the statistical significance of edges. We expected that the schizophrenia group would show greater randomisation.

2. We then repeated the analysis in (1), but at each density, we only included subjects where all edges satisfied $P_{FDR} < 0.01$ (i.e.: where all edges were significant by our
We expected the topological randomisation effect to disappear, implying that it is driven by inclusion of non-significant edges.

3. Finally, at each density, we subdivided both the schizophrenia and control groups into two subgroups: those subjects with non-significant edges (with $0.01 < P_{FDR} < 1$) at that density, and those without. Here we aimed to show definitively that it is inclusion of non-significant edges that drives the randomisation effect.

Group differences were evaluated using two-tailed Mann-Whitney U tests, and effect sizes using the simple difference formula (Kerby, 2014). For analyses (2) and (3) above, as group sizes varied as a function of density, we used permutation testing to ensure that our results were not driven by differences in statistical power. For analysis (2), we sampled $N$ subjects from each whole group (i.e.: controls or schizophrenia patients with all edge $P_{FDR} < 1$ at a given density) without replacement, where $N$ is the number of subjects used for the analysis at that density, and evaluated the effect size. For analysis (3), we shuffled group labels. In both cases, the permutation was run 10,000 times, and permutation-test $P$ values ($P_{perm}$) were calculated as the proportion of effect sizes of randomly sampled (or permuted) participants that exceeded the empirical effect size.

We repeated the above fixed-density analyses using values of efficiency and transitivity which were normalised with respect to their average values in 100 randomised networks with preserved degree distributions (Rubinov and Sporns, 2010), within each participant and at each density. Additionally, due to the previously reported relationships between group differences in mean correlation and topological measures (van den Heuvel et al., 2017), we evaluated differences in mean (unthresholded) correlation between subsets of participants whose edges were or were not significant at each density.

### 3.2.3 Topology in networks thresholded by probability or correlation

Having established, in Chapter 2, that a small proportion of edges differs between r- and $P$-thresholded connectomes, we wished to verify whether these lead to differences in topological organisation of the resulting networks. Therefore, we evaluated differences in global efficiency and transitivity between r- and $P$-thresholded networks, as a function of edge density. Within-group differences between topological measures derived from $P$ or r-based thresholds were evaluated using the Wilcoxon signed-rank test (WSR; a non-parametric test of paired sample differences). We repeated these analyses using values of efficiency and transitivity which were normalised relative to their average values in 100 randomised networks with preserved degree distributions (Rubinov and Sporns, 2010), for each thresholding method (increasing $P$ or decreasing r), within each participant and at each density.
3.3 Results

3.3.1 Integration and segregation in probabilistically-thresholded networks

We used probabilistic thresholding to address the hypothesis of topological randomisation in schizophrenia, by studying group differences in topological integration (global efficiency) and segregation (transitivity). We first thresholded all participants’ networks to fixed edge density by increasing P value, disregarding whether non-significant edges were included or not (but only considering participant at densities at which their connectomes contained edges with \( P_{FDR} < 1 \)). Consistent with the literature, patients with schizophrenia showed significantly increased efficiency, indicative of greater topological randomisation, across a range of densities (\( P_{MWU} < 0.05 \) at 2-35% density; Fig. 3.1A).

In the next analysis, at each density, we included only subjects where all edges met the criterion \( P_{FDR} < 0.01 \). In other words, we excluded subjects (at each density) whose connectomes contained non-significant edges. As expected, the difference in global efficiency between groups disappeared at nearly all densities (\( P_{MWU} < 0.05 \) at 5 and 7% density; Fig. 3.1B).

To identify whether it was indeed inclusion of non-significant edges which led to greater topological randomisation in schizophrenia, we subdivided each of our two groups into two further subgroups at each connection density: those that did not contain any non-significant edges (i.e.: all edge \( P_{FDR} < 0.01 \)), and those that did. The ratios of the size of these subgroups (as well as a third subgroup of participants whose remaining edges all display \( P_{FDR} = 1 \) and who were not considered in the analyses) are depicted in Fig. 3.1C and D (top row). We then compared the “significant” edge subgroup with the “non-significant” (or noisy) subgroup. We found that global efficiency was significantly increased in the group that contained non-significant edges compared to the group where all edges showed \( P_{FDR} < 0.01 \), across connection densities, for both healthy controls (both \( P_{MWU} \) and \( P_{perm} < 0.05 \) at 3-35% density; Fig. 3.1C) and patients with schizophrenia (\( P_{MWU} < 0.05 \) at 1-35% density, \( P_{perm} < 0.05 \) at 1% and 3-35% density; Fig. 3.1D). This suggests that it is in fact the inclusion of non-significant edges that artefactually inflates efficiency, through increased randomisation.

These results were generally consistent when normalised with respect to random networks (where values of randomised efficiency tend to 1, as expected). Although differences in efficiency between full groups (participants with all edge \( P_{FDR} < 1 \)) were reduced relative to un-normalised efficiency (\( P_{MWU} < 0.05 \) at 22% and 33% edge density), subsets of non-significant participants showed substantial decreases in normalised efficiency relative to...
Fig. 3.1 Effects on global efficiency of adding non-significant edges to functional connectomes. A) Global efficiency as a function of edge density, in all participants with edge \( P_{\text{FDR}} < 1 \) at each density (regardless of edge significance). B) Global efficiency as a function of edge density in the subset of participants whose edges are all significant at each density (left axis). The number of participants compared decreases as a function of edge density (right axis). C,D) Top: Density-dependent proportion of significant and non-significant participants, for healthy controls (C), and patients with schizophrenia (D). Bottom: Global efficiency as a function of edge density, between significant and non-significant healthy controls (C) and patients with schizophrenia (D). Black dots indicate \( P_{\text{MWU}} < 0.05 \), black circles indicate permutation test \( P_{\text{perm}} < 0.05 \). Thick lines indicate medians; shaded lines indicate quartiles.
3.3 Results

Fig. 3.2 **Effects on transitivity of adding non-significant edges to functional connectomes.** A) Transitivity as a function of edge density, in all participants with edge $P_{FDR} < 1$ at each density (regardless of edge significance). B) Transitivity as a function of edge density, in the subset of participants whose edges are all significant at each density (left axis). The number of participants compared decreases as a function of edge density (right axis). C,D) Top: Density-dependent proportion of significant and non-significant participants, for healthy controls (C), and patients with schizophrenia (D). Bottom: Transitivity as a function of edge density, between significant and non-significant healthy controls (C) and patients with schizophrenia (D). Black dots indicate $P_{MWU} < 0.05$, black circles indicate permutation test $P_{perm} < 0.05$. Thick lines indicate medians; shaded lines indicate first and third quartiles.

Patients with schizophrenia showed decreased transitivity at higher edge densities ($P_{MWU} < 0.05$ at 20-23% and 32-35% density) when comparing all participants with $P_{FDR} < 1$ (Fig. 3.2A). These differences disappeared when comparing subsets of significant participants (all $P_{MWU}$ and $P_{perm} > 0.05$; Fig. 3.2B). Subsequently, transitivity was lower in the non-significant healthy controls ($P_{MWU}$ and $P_{perm} < 0.05$ at 7-25% and 28-35% density; Fig. 3.2C), and in non-significant patients with schizophrenia ($P_{MWU}$ and $P_{perm} < 0.05$ at 1% and 12-35% density; Fig. 3.2D).
Conversely, when normalised relative to random networks, patients with schizophrenia exhibited higher transitivity than healthy controls when comparing all participants with $P_{\text{FDR}} < 1$ ($P_{\text{MWU}} < 0.05$ at 1-34% density). These differences were reduced within subsets of significant participants ($P_{\text{MWU}} < 0.05$ at 1-6% density). Furthermore, non-significant participants displayed higher transitivity than significant ones, both within healthy controls ($P_{\text{MWU}} < 0.05$ at 7-25% and 28-35%, $P_{\text{perm}} < 0.05$ at 2-35% density) and patients with schizophrenia ($P_{\text{MWU}} < 0.05$ at 1% and 12-35% density, $P_{\text{perm}} < 0.05$ at 1-35% density).

### 3.3.2 Group differences in network disconnectivity and mean correlation

As discussed, higher-order measures such as efficiency and transitivity, explored here in the context of the topological randomisation hypothesis of schizophrenia, may themselves be dependent on simpler network properties. Therefore, we repeated the above analyses using the number of connected components, and the mean unthresholded correlation (of subsets of 'significant' or 'non-significant' participants at each density).

When all participants (with all edge $P_{\text{FDR}} < 1$) were compared, patients with schizophrenia showed a lower number of (larger) connected components, across densities ($P_{\text{MWU}} < 0.05$ at 1-30% and 32% density; Fig. 3.3A). However, when subsets of significant participants were compared, these differences were less extensive ($P_{\text{MWU}} < 0.05$ at 1-10% density; Fig. 3.3B). Accordingly, non-significant participants displayed a lower number of (smaller) connected components than significant participants, both within healthy controls (both $P_{\text{MWU}}$ and $P_{\text{perm}} < 0.05$ at 3-35% density; Fig. 3.3C) and within patients with schizophrenia (both $P_{\text{MWU}}$ and $P_{\text{perm}} < 0.05$ at 1-35% density; Fig. 3.3D).

Finally, we observed differences in mean correlation (of unthresholded networks) between healthy controls and patients with schizophrenia (comparing only connectomes of participants with all edge $P_{\text{FDR}} < 1$ at a given density) across edge densities ($P_{\text{MWU}} < 0.05$ at 1-27%, 29-30% and 33-35% density; Fig. 3.4A). These effects largely disappeared when only subsets of significant participants were compared ($P_{\text{MWU}} < 0.05$ at 1-5% density; Fig. 3.4B). Further, non-significant participants displayed lower mean correlation, both within healthy controls (both $P_{\text{MWU}}$ and $P_{\text{perm}} < 0.05$ at 2-35% density; Fig. 3.4C) and patients with schizophrenia (both $P_{\text{MWU}}$ and $P_{\text{perm}} < 0.05$ at 1-35% density; Fig. 3.4D).
3.3 Results

Fig. 3.3 **Effects on the number of connected components of adding non-significant edges to functional connectomes.** A) The number of connected components as a function of edge density, in all participants with edge $P_{\text{FDR}} < 1$ at each density (regardless of edge significance). B) The number of connected components as a function of edge density, in the subset of participants whose edges are all significant at each density (left axis). The number of participants compared decreases as a function of edge density (right axis). C,D) Top: Density-dependent proportion of significant and non-significant participants, for healthy controls (C), and patients with schizophrenia (D). Bottom: The number of connected components as a function of edge density, between significant and non-significant healthy controls (C) and patients with schizophrenia (D). Black dots indicate $P_{\text{MWU}} < 0.05$, black circles indicate permutation test $P_{\text{perm}} < 0.05$. Thick lines indicate medians; shaded lines indicate first and third quartiles.
Fig. 3.4 **Mean correlation in subsets of significant and non-significant participants.**

A) The mean (unthresholded) correlation as a function of edge density, in all participants with edge $P_{FDR} < 1$ at each density (regardless of edge significance). B) The mean correlation as a function of edge density, in the subset of participants whose edges are all significant at each density (left axis). The number of participants compared decreases as a function of edge density (right axis). C,D) Top: Density-dependent proportion of significant and non-significant participants, for healthy controls (C), and patients with schizophrenia (D). Bottom: The mean correlation as a function of edge density, between significant and non-significant healthy controls (C) and patients with schizophrenia (D). Black dots indicate $P_{MWU} < 0.05$, black circles indicate permutation test $P_{perm} < 0.05$. Thick lines indicate medians; shaded lines indicate first and third quartiles.
3.3 Results

Fig. 3.5 Differences in topology between networks thresholded by decreasing correlation or increasing P value. A) Within-participant differences in efficiency between connectomes thresholded by increasing P and decreasing r. Plots represent the median difference in efficiency across participants. Efficiency is generally increased in connectomes thresholded by r (compared to those thresholded by P), in both healthy controls (i) and patients with schizophrenia (ii). B) Within-participant differences in transitivity between connectomes thresholded by increasing P and decreasing r. Plots represent the median difference in transitivity across participants. Transitivity is increased in connectomes thresholded by r (compared to those thresholded by P) at low edge densities, and increased at higher edge densities, in both healthy controls (i) and patients with schizophrenia (ii).

3.3.3 Differences in topology between r- and P-thresholded connectomes

Furthermore, we evaluated within-subject differences in measures of topological organisation between connectomes thresholded using increasing P and decreasing r. Global efficiency was weakly increased in r-thresholded networks relative to P-thresholded networks at a range of edge densities in both healthy controls (P_{WSR} < 0.05 at 4-35% density, Fig. 3.5Bi), and patients with schizophrenia (P_{WSR} < 0.05 at 2-35% density, Fig. 3.5Bii). Transitivity was lower in P-thresholded networks at low edge densities in both healthy controls (P_{WSR} < 0.05 at 1-5% density, Fig. 3.5Ci) and patients with schizophrenia (P_{WSR} < 0.05 at 2% density, Fig. 3.5Cii). However, at higher edge densities, transitivity was increased in P-thresholded networks relative to correlation-thresholded networks, in both healthy controls (P_{WSR} < 0.05 at 10-35% density) and in patients with schizophrenia (P_{WSR} < 0.05 at 10-35% density).
Results were fully consistent following normalisation of topological measures in \( r \)- and \( P \)-thresholded connectomes by corresponding average values from sets of 100 randomised networks with preserved degree distributions, leading to significant group differences of the same sign at the same edge densities.

### 3.4 Discussion

In this chapter, we confirm that comparison of functional connectomes with different weight distributions (such as patients with schizophrenia and controls) using arbitrary weight-based thresholds risks introduction of “non-significant” false positive edges and thus group differences in topological organisation (Fornito et al., 2012; van den Heuvel et al., 2017). The probabilistic thresholding methods we propose to mitigate this risk are statistically principled and simple to implement.

Moreover, we explicitly compare the application of thresholding connectomes by \( P \) value (following adjustment for effective \( df \)) to existing thresholding methods based on correlation (equivalent to assuming nominal \( df \)), demonstrating that the retention of different edges leads to differences in topology.

#### 3.4.1 Topological randomisation and schizophrenia

A number of studies have reported increased topological randomisation of functional connectomes in patients with schizophrenia (Rubinov et al., 2009; Alexander-Bloch et al., 2010; Lynall et al., 2010; Lo et al., 2015). However, as suggested by Fornito et al. (2012), this was likely a consequence of applying fixed-density thresholds to groups with different edge weight distributions. Methodologies such as those we propose here, where cohorts are matched by connectivity probabilities, can be used to control for this.

When using traditional density thresholding, comparing cohorts regardless of differences in weight distributions, we found increased global efficiency in patients with schizophrenia relative to controls. With progressive randomisation, the path length of a graph decreases (Watts and Strogatz, 1998) while its global efficiency increases (Latora and Marchiori, 2001); this finding therefore aligns with previous reports of increased topological randomisation in schizophrenia. However, this effect disappears when controlling cohorts for type I error, i.e.: when excluding connectomes which contain edges with probabilities \( P_{\text{FDR}} > 0.01 \) (and thus non-significant edges) from analysis. We further show, by comparing within each cohort connectomes with non-significant edges and those without, at a range of edge densities, that differences in global efficiency are driven by inclusion of non-significant edges. In other
words, inclusion of noise increases efficiency and produces a more random topology. These findings remained qualitatively consistent when normalising with respect to random networks with preserved degree distributions (Rubinov and Sporns, 2010).

Together, these findings confirm the hypothesis advanced by Fornito et al. (2012) that increased randomisation in schizophrenia results from correlation-based thresholding to fixed edge density. At a lower level, these differences seem to be driven by a noise-driven “coalescence” of the fixed-density patient connectomes relative to healthy ones. When all participants are compared (regardless of edge significance), patients with schizophrenia exhibit lower numbers of (larger) connected components, in line with a previous study using fixed-density correlation-based thresholding (Bassett et al., 2012). However, these differences are substantially less extensive when only connectomes with edge $P_{FDR} < 0.01$ are included in analysis. Accordingly, connectomes with non-significant edges exhibit lower numbers of (larger) connected components. As the topological path length between pairs of disconnected nodes is infinite, the corresponding contribution to efficiency (which depends inversely on the path length) will be null, and the global efficiency will decrease (Fornito et al., 2016). The lower number of connected components in non-significant connectomes suggests that randomly placed non-significant edges are likely to act as “bridges” between disparate clusters of significant edges.

In line with previous reports of decreased segregation in functional connectomes of patients with schizophrenia, reported as decreased local efficiency (Liu et al., 2008) and clustering in both patients (Alexander-Bloch et al., 2010; Lynall et al., 2010) and their siblings (Lo et al., 2015), we found decreased transitivity (a global measure of segregation) in patients - although only at certain edge densities. Still, general decreases in transitivity in non-significant connectomes (both within healthy controls and patients with schizophrenia) suggest that the addition of noisy edges increases topological randomness by decreasing transitivity. When normalising with respect to random networks with preserved degree distributions, we found increased normalised transitivity in patients relative to controls, and in connectomes with non-significant edges relative to those without non-significant edges. These disparities between our findings and the literature might be explained by the application of different measures – whereas the widely used average clustering coefficient has a dependence on the degree distribution of the underlying graph, transitivity does not (Newman, 2010). Furthermore, we can speculate that transitivity might be less affected by noisy edges than efficiency, as the addition of a single “noisy” edge will affect all paths traversing that edge, whereas it will only affect transitive closure in the immediate vicinity of the nodes it is connected to.
Our findings linking traditional weight-based fixed-density thresholding to topological randomisation align with a recent study by van den Heuvel et al. (2017), which demonstrates that group differences in overall functional connectivity strength lead to group differences in topological organisation of fixed-density connectomes, across several empirical and simulated case-control datasets. We note a relationship between our experiments and those presented in van den Heuvel et al. (2017): when comparing subsets of significant participants to ensure control over type I error, the difference in mean correlation between healthy controls and patients with schizophrenia is reduced. Subsequently, subsets of significant and non-significant participants show large differences in mean correlation. van den Heuvel et al. (2017) postulate possible strategies to control for effects of group differences in average functional connectivity, including by (i) regressing mean connectivity from graph-theoretical measures, or (ii) comparing empirical differences in network measures to a null distribution, built by permuting group labels such that empirical differences in mean connectivity are preserved (van den Heuvel et al., 2017).

Here we describe a statistically-reasoned approach to limit inclusion of non-significant edges, which may result in artefactual group differences in topological organisation. This methodology, based on the probabilistic connectivity method described in Patel and Bullmore (2016), generates estimates of effective $df$ from robust denoising of motion artefacts by wavelet despiking (Patel et al., 2014). To the best of our knowledge, these wavelet-based methods are the only currently available means of estimating the spatial and temporal variability in loss of confidence in time series recorded from motion-affected brain areas. As discussed in Patel and Bullmore (2016) and as demonstrated here on a dataset of healthy controls and patients with schizophrenia, this loss of effective $df$ has substantial effects on the estimates of topological organisation of functional connectomes.

### 3.4.2 Further considerations

Analyses presented herein are methodological in nature. Studies presented in Chapter 2 and in this chapter focus on the implication of network thresholding approaches for the estimation of summary measures of network organisation, and subsequent between-group comparisons. Schizophrenia was analysed as a model disease presenting reduced functional correlation distributions, and as an example of hypothetical "network randomisation" (Fornito et al., 2012, 2013). This methodological focus limits our ability to make strong claims about neurophysiological alterations in schizophrenia; nevertheless, lessons for future investigation of this (and other) diseases may be drawn, concerning the need to understand the root cause of group differences.
While fMRI studies may only identify macroscopic signatures of underlying microscopic neurobiological alterations in schizophrenia (Kahn and Sommer, 2015), it is important that the differences reported by fMRI studies are as fundamental as possible, by being disentangled into components that are maximally independent of preceding steps. Thus, if previously reported differences in network randomisation were methodologically rooted in the thresholding of shifted correlation distributions to fixed edge density, it is preferable to report these underlying shifts in correlation distribution. In turn, such shifts may be linked to alterations in properties of the time-series used to estimate functional correlations, including the power (Zalesky et al., 2012; Yang et al., 2014) and entropy (Yang et al., 2015) of the BOLD signal. Similarly, variations in global connectivity across subjects should be taken into account when investigating regional differences between groups. In Chapter 2, we found wide-spread differences in node degree (of networks thresholded based on fixed P value); however, these differences disappeared when the variable edge density of individual participants’ networks was taken into account.

Methods presented herein shed light on previously claimed differences in network randomisation. It seems likely, based on our results and those of van den Heuvel et al. (2017), that the previously reported differences were methodologically grounded in the thresholding of networks to fixed edge density based on correlation – and we suggest that this can be avoided by using the methods presented in this chapter and in Chapter 2. Importantly, we do not claim that there are definitely no differences in topological organisation between patients with schizophrenia and healthy controls - but such differences might be subtle, and their identification will likely be trickier than previously thought.

### 3.4.3 Conclusion

In summary, probabilistic thresholding methods presented in this section of this thesis confirm greater disconnectivity in schizophrenia, with fewer significant connections between regions. Thresholding to fixed edge density (by effective-\(df\)-adjusted P value) supports the view that previously reported increases in randomisation within functional connectomes in schizophrenia were linked to the inclusion of a greater proportion of noisy edges within patients’ connectomes, which exhibit shifted correlation distributions. Finally, while only a small proportion of edges differ between functional connectomes thresholded to fixed edge density based on edge weights and P values, these edges may lead to significant alterations in global topological organisation.

As discussed in Chapter 2, application of the probabilistic thresholding methods presented herein is contingent on estimation of subject-specific, spatially heterogeneous estimates of effective \(df\) of regional neurophysiological time-series. To the best of our knowledge,
spatially heterogeneous loss of $df$ due to motion artefacts, and resulting adjustment of correlation probabilities, can currently only be estimated using wavelet despiking methods (Patel et al., 2014; Patel and Bullmore, 2016).

Our results warrant care during analysis of connectomes constructed using correlations, and emphasise the need for exploration of potential “lower-order” causes (such as shifts in edge weight distributions between groups or across participants) of alterations in “higher-order” topological properties of brain network organisation.
PART II

SLIDING WINDOW CONSTRUCTION OF STRUCTURAL NETWORKS
4.1 Introduction

Human adolescence is known to be a major phase of cortical development. In particular, cerebral cortex becomes thinner (Wierenga et al., 2014) and more densely myelinated (Miller et al., 2012) in the transition from puberty to young adulthood. Adolescent decreases in cortical thickness (thinning) are variable between different areas of cortex (Raznahan et al., 2011): for example, thinning is greater in association cortical areas than primary sensory areas (Whitaker et al., 2016).

Motivated by these and other results, we predicted that human adolescence should be associated with changes in the architecture of structural brain networks. There are currently only two experimental techniques, both based on magnetic resonance imaging (MRI), that are capable of providing data to test this prediction: diffusion tensor imaging followed by tractography; or structural MRI followed by structural covariance or correlation analysis. Here we focused on the latter, measuring the thickness of a set of predefined cortical regions in each individual MRI dataset and then estimating the correlation of thickness between each possible pair of regions across participants. Similar methods have been widely used and validated (Lerch et al., 2006) in a range of prior studies (Alexander-Bloch et al., 2013a; Evans, 2013).

In particular, structural correlation (covariance) measures have been used as a basis for graph theoretical modelling of the human connectome (Bullmore and Sporns, 2009; Fornito et al., 2016). Considerable evidence has accumulated in support of the general view that human brain structural correlation networks have a complex topological organization, characterised by non-random features such as the existence of highly connected (high degree) hub nodes and a modular community structure (Alexander-Bloch et al., 2013a; Evans, 2013).
Topological metrics on structural correlation networks have demonstrated changes associated with disease, development and ageing (Alexander-Bloch et al., 2013a; Evans, 2013). However, only two studies have investigated adolescent changes in structural correlation networks. Zielinski et al. (2010) demonstrated that the anatomical extent of structural correlation networks, assessed using seed-based correlation of voxel-wise grey matter intensity, changes in adolescence in a spatially patterned manner. Specifically, primary visual and sensori-motor networks, as well as the default mode network, expanded in early childhood before being “pruned” in adolescence, while higher-order cognitive networks showed a gradual monotonic gain in spatial extent. Subsequently, Khundrakpam et al. (2013) applied graph-theoretical analyses to a subset of the same data, reporting childhood increases in topological integration (global efficiency) and decreases in topological segregation (local efficiency and modularity), as well as increases in regional integration in paralimbic and association regions. For a summary of these and related studies, see Table A.1 in Appendix A. While these studies constitute interesting initial investigations, their ability to precisely describe developmental changes is limited by their segregation of participants into four discrete age-defined strata, resulting in relatively coarse-grained resolution of brain maturational trajectories.

Here, we aimed to obtain a more precise description of adolescent maturational trajectories of structural network architecture, which were hypothesised to vary as a smooth and potentially non-linear function of age. We used a sliding-window analysis to estimate structural correlations and structural network properties for each of an overlapping series of 9 age-defined windows or strata of the sample (N ~ 60 participants per window). We identified the cortical regions (nodes) and connections (edges) which showed the most significant age-related changes in structural correlation. We tested the related hypotheses that parameters of adolescent change in structural correlation would be greater and occur later in regions of association cortex, which show faster rates of local cortical shrinkage and myelination. In addition, we explored whether greater and later changes in structural correlation during adolescence would be concentrated within or between specific communities of regions. Specifically we mapped adolescent changes in structural correlation to three brain community structures: the topological modular partition of the age-invariant structural correlation network; an atlas of cytoarchitectonic classes (von Economo and Koskinas, 1925); and functional intrinsic connectivity or resting state networks (Yeo et al., 2011).
4.2 Methods

4.2.1 Participants

A demographically balanced cohort of 297 healthy participants (149 females) aged 14-24 years was included in this study, with approximately 60 participants in each of 5 age-defined strata: 14-15 years inclusive, 16-17 years, 18-19 years, 20-21 years, and 22-24 years. Participants were excluded if they were currently being treated for a psychiatric disorder or for drug or alcohol dependence; had a current or past history of neurological disorders or trauma; or had a learning disability. Participants provided informed written consent for each aspect of the study, and parental consent was obtained for those aged 14–15 years. The study was ethically approved by the National Research Ethics Service and was conducted in accordance with NHS research governance standards.

4.2.2 MRI acquisition and processing

Structural scans were acquired at three sites using multi-parametric mapping (MPM) implemented on three identical 3T MRI scanners (Siemens Magnetom TIM Trio). Inter-site reliability of the sequence was evaluated within a pilot study of five healthy participants each scanned at each site (Weiskopf et al., 2013). The MPM sequence includes maps of $R_1 (1/T_1)$ and magnetization transfer (MT), indicative of myelination.

The following details concerning the multi-parametric mapping (MPM) sequence have previously been described by Weiskopf et al. (2013) and Whitaker et al. (2016). The MPM sequence comprised three multi-echo 3D FLASH (fast low angle shot) scans, with predominant weighting determined by choice of the repetition time (TR) and the flip angle $\alpha$: for the $T_1$ scan, TR = 18.7 ms, $\alpha = 20^\circ$; for the MT scan, TR = 23.7 ms, $\alpha = 6^\circ$. Multiple gradient echoes were acquired with alternating readout polarity at six equidistant echo times (TE) between 2.2 and 14.7 ms for both acquisitions. Other acquisition parameters were: 1 mm isotropic resolution, 176 sagittal partitions, field of view (FOV) = 256 × 240 mm, matrix = 256 × 240 ×176, parallel imaging using GRAPPA factor 2 in phase-encoding direction (AP), 6/8 partial Fourier in partition direction, non-selective RF excitation, readout bandwidth = 425 Hz/pixel, RF spoiling phase increment = 50°. The total acquisition time was ~25 min. Participants were given standard ear protection and instructed to lie still and rest during the scan.

Processing of individual scans using FreeSurfer v5.3.0 included skull-stripping, segmentation of cortical grey and white matter and reconstruction of the cortical surface and grey-white matter boundary (Fischl et al., 1999). All FreeSurfer reconstructions were visu-
Adolescent tuning of association cortex in structural brain networks

ally inspected and manually edited by members of the Neuroscience in Psychiatry Network (NSPN) Consortium. The recon-all algorithm was run after each round of manual quality control and up to 10 iterations of edits were performed. Complete cortical reconstruction was required for data to be included in the final analysis. There were 320 scans collected, with 23 scans excluded as they did not meet these quality requirements. The cerebral cortex of each participant was parcellated into 308 regions of interest, based on a sub-division of the Desikan-Kiliany anatomical atlas (Desikan et al., 2006) into parcels of approximately equal surface area (~5cm²; Romero-Garcia et al., 2012).

4.2.3 Rates of thinning and myelination

Regional changes in cortical thickness (CT) and myelination (MT) were characterized using the rate of change over adolescence, evaluated as the slope of a linear model fitted to the cross-sectional values. Following Whitaker et al. (2016), myelination analyses were conducted at 10 fractional depths between the pial surface and the grey/white matter boundary, as well as two absolute depths into white matter. The 10 intra-cortical samples of MT were chosen as fractions of cortical depth to adjust for differing cortical thicknesses between regions. For depths below the grey-white matter boundary, absolute depths were used to maintain uniformity across regions. The values of 0.4 mm and 0.8 mm were chosen for their correspondence to the spacing used for intra-cortical samples in the thickest cortical regions. Main analyses focused on MT estimates at 70% fractional cortical depth from the pial surface. For details on the extraction of MT values at variable cortical depths, see Whitaker et al. (2016).

While both cortical thickness and myelination maps were averaged within parcels, for comparison between maturation of structural correlation networks and morphology, only the cortical thickness values were used to construct structural correlation networks.

4.2.4 Age-invariant structural network

An age-invariant structural correlation network was constructed using Pearson correlations in cortical thickness between pairs of regions across all 297 participants, to serve as a reference for developmental changes within the age-resolved structural networks (described below; Fig. 4.1A). We used raw cortical thickness values, uncorrected for age, gender or intra-cranial volume. However, correcting for these covariates had no effect on the results.

The age-invariant structural network was thresholded using a bootstrap approach, whereby 1000 sets of participants were resampled with replacement and used to construct surrogate structural networks. For each pair of regions, we examined whether there is evidence of a non-
zero correlation across bootstraps: edges that were consistently positive or negative across bootstraps (at a two-tailed, FDR-adjusted level of $\alpha = 0.01$) were retained; the remaining edges were set to zero. Nodal topological organisation of the thresholded network was assessed using degree, defined as the number of retained correlations for each node, as well as the weighted degree, or summed weight of retained edges for each node.

Further, the age-invariant network was partitioned into communities of nodes showing higher structural correlations within than between communities (Sporns and Betzel, 2016). The community structure of the age-invariant network was decomposed using the Louvain multi-resolution algorithm (Blondel et al., 2008) over the resolution parameter range $0.01 \leq \gamma < 4.00$. As $\gamma$ increases, the community structure is decomposed to a progressively larger number of modules. We used the concept of minimizing versatility to identify those resolution parameter values which reduce the uncertainty with which any node was affiliated consistently to the same module (Shinn et al., 2017). The final community partition was defined as a consensus across 1000 runs of the Louvain modularity algorithm (Lancichinetti and Fortunato, 2012) at the selected value of the resolution parameter $\gamma$.

4.2.5 Development of age-resolved structural networks

Sliding window network construction

Development of structural networks between 14 and 24 years was evaluated using a sliding window method. Regional cortical thickness values were cross-correlated within windows containing equal numbers of participants, and incrementally slid across the age-range by regular steps (Fig. 4.1B). The two parameters of the method, the “window width” and the “step size” (in units of number of participants) determine the number of windows, each of which generates a structural correlation network. Exploration of the sliding window parameter values suggests that results are qualitatively consistent across a range of parameter combinations. For the (in)dependence of results on sliding window parameters, and a discussion of the considerations involved in parameter selection, see Chapter 5.

Results presented below correspond to nine half-overlapping windows of 60 participants each, obtained by interpolating the five age strata of the NSPN study, within which participants were recruited. Gender was relatively balanced within the interpolated bins, with the most imbalanced ratio being $34:26 = 57\%:43\%$ (M:F). We investigated the effects of gender separately (see Chapter 5).

Global maturation of structural networks was characterised using the mean of the correlation distribution. At the regional level, an analogous measure was used – nodal strength,
the mean of the pattern of regional correlations (rows, or equally, columns of the correlation matrices).

**Bootstrap thresholding of age-resolved structural networks**

Estimating structural correlation networks from a small number of participants is an inherently noisy process; therefore, our principal analyses focused on networks probabilistically thresholded using bootstrap (Fig. 4.5B). The bootstrap thresholding procedure was identical to the one described above for age-invariant networks, but in this case was applied within windows. From the set of participants included in each window, an equal number of participants was sampled with replacement and the correlation structure was re-estimated 1000 times. For each pair of regions, we examined whether there is evidence of a non-zero correlation across bootstraps: edges that were consistently positive across bootstraps (at a two-tailed, FDR-adjusted level of $\alpha = 0.01$) were retained (there were no consistently negative edges); the remaining edges were set to zero.

The global topological organisation of the thresholded graphs was assessed using the edge density, defined as the percentage of retained edges (relative to their total possible number), as well as the distance spanned by retained edges, calculated as the average Euclidean distance between centroids of corresponding nodes. Nodal topological organisation was assessed using (analogous) measures of degree, defined as the number of edges connected to a node, and average Euclidean distance spanned by a node’s retained edges. We have focused on simple graph-theoretical measures, such as edge density and node degree, for two reasons: first, our bootstrap-thresholded networks display variable edge density, which many “higher-order” graph-theoretical measures show a strong dependence on (van Wijk et al., 2010); and second, even in correlation-based networks thresholded to fixed edge density, graph theoretical properties display a dependence on more elementary statistics such as properties of the correlation distribution (van den Heuvel et al., 2017).

### 4.2.6 Fitting and characterisation of developmental trajectories

Developmental trajectories were fitted to both global and local measures as a function of the median age of participants in each window. In addition to linear models, we fitted locally adaptive smoothing splines. The nonparametric smoothing spline was chosen to model nonlinear trajectories over parametric alternatives as it was shown to be superior to quadratic fits in studies of brain development (Fjell et al., 2010). Still, the spline fits were constrained to be (approximately) at least as smooth as a quadratic fit (i.e.: effective degrees of freedom, $df \leq 3.5$), based on the hypothesis that adolescent developmental trajectories
Fig. 4.1 **Construction of age-invariant and age-resolved structural correlation networks.** A) An age-invariant structural correlation network was constructed by cross-correlating regional cortical thickness across all participants. This network was probabilistically thresholded using a bootstrap-based method. Network organisation was evaluated using several measures, including the degree (both binary and weighted, respectively the number and sum of weights of retained edges connected to a node) and modular architecture. B) Age-resolved structural correlation networks were constructed using a sliding-window method. Participants were ordered by age, and structural networks were constructed by estimating correlations between regional cortical thickness values across participants within overlapping windows iteratively slid across the age range. Correlations were probabilistically thresholded using bootstrap, before developmental trajectories were fitted to summary window-derived measures as a function of the median age of participants within each window.
over a 10-year age range should not display greater complexity. The specific smoothing spline used was a weighted sum of 6 cubic b-splines with knots placed at quantiles of the data and smoothing optimised using restricted maximum likelihood (REML; Reiss et al., 2014). The relative quality of linear and spline fits, given their parsimony, was assessed using Akaike’s information criterion (AIC, denoted as $AIC_{\text{lin}}$ and $AIC_{\text{spl}}$ for linear models and smoothing splines respectively). Classification using the Bayesian Information Criterion (BIC) yielded consistent results.

Regional changes were summarised using measures of maximum change in degree $\Delta k_{\text{max}}$, quantified as the difference between maximum and minimum degree, and the age at minimum degree age($k_{\text{min}}$). Further, we classified regional changes in degree as linear or non-linear (using the AIC), and as increasing or decreasing (using the direction of maximum change).

### 4.2.7 Relationship of network development to age-invariant architecture

Given our previous finding, that highly correlated “hub nodes” of the age-invariant structural network (derived from all participants) are regions which thin and myelinate most over adolescence (Whitaker et al., 2016), we were interested in studying the relationship of structural network development to age-invariant structural network architecture.

We evaluated Spearman’s rank correlations between node degree in the age-invariant structural network, and parameters of change in node degree within the age-resolved structural network – including the amplitude of maximum change in degree $\Delta k_{\text{max}}$ as well as the age at minimum degree age($k_{\text{min}}$).

Finally, we studied changes in structural network organisation relative to three sets of node communities, including the partition of the age-invariant network into modules, the von Economo atlas of cytoarchitectonic classes (von Economo and Koskinas, 1925), and a set of functional intrinsic connectivity networks (Yeo et al., 2011) (described in greater detail below). For each community template and each age-window, we calculated the local density of edges $D$ within each community as well as between each pair of communities (within the same template), as the ratio of existing edges relative to the maximum number of possible edges in this within or between-community edge set. We then characterised changes in edge density within and between communities using measures analogous to the nodal trajectories – maximum change in edge density $\Delta D_{\text{max}}$ and age at minimum density age($D_{\text{min}}$).
4.2 Methods

**Fig. 4.2** *Surface maps and community labels for two independent community templates.* A) Cytoarchitectonic classes of the von Economo atlas (von Economo and Koskinas, 1925). B) Functional intrinsic connectivity networks (Yeo et al., 2011).

**The von Economo atlas of cytoarchitectonic classes**

The classification of regions into cytoarchitectonic classes was conducted manually based on the five original subtypes described by von Economo and Koskinas (1925); in order of increasing laminar differentiation, these correspond to primary motor cortex (structural type 1), association cortex (structural types 2 and 3) and secondary and primary sensory cortex (types 4 and 5). Our classification further distinguishes two additional subtypes - limbic regions and insular cortex, neither of which are easily assigned to the five original classes (Mai and Paxinos, 2012), resulting in seven cytoarchitectonic subtypes (Vértes et al., 2016). For a surface map with class labels, see Fig. 4.2A.

**Functional intrinsic connectivity networks**

We studied trajectories of structural network development relative to an independent cortical parcellation based on seven functional connectivity networks (Yeo et al., 2011). We assigned the 308 regions in our parcellation to one of the seven classes based on the greatest proportion of overlap of each region to each class. The proportion of overlap per region was calculated in the Freesurfer *fsaverage* template volume as the number of voxels within each network, divided by the total number of voxels. For a surface map with network labels, see Fig. 4.2B.
4.2.8 Spatial permutation test

In several analyses in the current study, measures were related to each other across regions. While numerous studies have reported significance based on the assumption that the number of samples is equal to the number of regions, this is technically inaccurate, as the number of regions is both arbitrary (due to the resolution of the chosen parcellation) and non-independent (due to spatial auto-correlation amongst neighbouring parcels). To address this issue, spatial permutation tests have been implemented in past studies (Alexander-Bloch et al., 2013b; Vandekar et al., 2015), which consist in comparing the empirical correlation amongst two spatial maps to a set of null correlations, generated by randomly rotating the spherical projection of one of the two spatial maps (as generated in FreeSurfer or Caret) before projecting it back on the brain surface. Importantly, the rotated projection preserved spatial contiguity of the empirical maps, as well as hemispheric symmetry. Such tests were previously implemented at the vertex level (Alexander-Bloch et al., 2013b; Vandekar et al., 2015); here we implemented an analogous permutation test at the regional level.

The spatial permutation test was implemented in the following manner: First, we obtained the coordinates of each of our 308 regions on the FreeSurfer spherical projection of the parcellation. We next rotated these coordinates about the three axes (x: left-right, y: rostral-caudal, z: dorsal-ventral) at three randomly generated angles, $\theta_x$, $\theta_y$ and $\theta_z \in [0,2\pi)$, using the following rotation matrices:

$$R_x(\theta) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos(\theta) & -\sin(\theta) \\ 0 & \sin(\theta) & \cos(\theta) \end{bmatrix} \quad (4.1)$$

$$R_y(\theta) = \begin{bmatrix} \cos(\theta) & 0 & \sin(\theta) \\ 0 & 1 & 0 \\ -\sin(\theta) & 0 & \cos(\theta) \end{bmatrix} \quad (4.2)$$

$$R_z(\theta) = \begin{bmatrix} \cos(\theta) & -\sin(\theta) & 0 \\ \sin(\theta) & \cos(\theta) & 0 \\ 0 & 0 & 1 \end{bmatrix} \quad (4.3)$$

Since each hemisphere is projected onto the sphere separately, the rotation was applied to both hemispheres. To preserve hemispheric symmetry, the same random angles were applied to both hemispheres, with the caveat that the sign of the angles was flipped for the rotations around the y and z axes; i.e., $\theta_yR = -\theta_yL$ and $\theta_zR = -\theta_zL$ (but $\theta_xR = \theta_xL$).
4.2 Methods

Fig. 4.3 Demonstration of the spatial permutation test. The spatial permutation test was applied three times to maps of the x, y and z coordinates of regional centroids in MNI space. The spatial contiguity and hemispheric symmetry is preserved within the permuted maps.

Following rotation of the sphere, coordinates of the rotated regions were matched to coordinates of the initial regions, using Euclidean distance and proceeding in a descending order of average Euclidean distance between pairs of regions on the rotated and unrotated spheres (i.e.: starting with the rotated region that is furthest away, on average, from the unrotated regions). The matching then provides a mapping from the set of regions to itself, that allows any regional measure to be permuted while controlling for spatial contiguity and hemispheric symmetry.

For an example of outputs of the permutation test, applied to smooth maps of the \{x,y,z\} centroids of regions in MNI space, see Fig. 4.3.

P values for the correlation between two maps were obtained by comparing the empirical value of Spearman’s \( \rho \) to a null distribution of 10’000 Spearman correlations, between one empirical map and a set of 10’000 spatially permuted versions of the other map. Each analysis correlating values from two cortical maps is reported with both the P value corresponding to the Spearman correlation (\( P_{\text{Spearman}} \)), as well as a P value derived from the spherical permutation (\( P_{\text{perm}} \)).
4.3 Results

4.3.1 Age-invariant structural network

We first considered the structural correlation network constructed by thresholding the pairwise inter-regional correlations estimated from cortical thickness measurements on all (297) participants, age range 14-24 years (inclusive). Since this analysis combines data from all ages in the sample, we can refer to the result as an age-invariant structural correlation network (Fig. 4.1A).

The distribution of structural correlations had a positive mean value and was approximately symmetrical. The structural correlation matrix was thresholded probabilistically, using a bootstrap-based resampling procedure (Methods), to control the edge-wise false positive rate. Since this thresholding operation entailed approximately 47,000 hypothesis tests, we used the false discovery rate (FDR) algorithm to adjust for multiple comparisons. The resulting graph was densely connected (connection density $\approx 90\%$) and exhibited a modular community structure (Figs. 4.1A, 4.4).

Module decomposition of the age-invariant structural correlation network

To decompose the bootstrap-thresholded (but weighted) age-invariant structural correlation network into modules using the Louvain multi-resolution algorithm, we identified values of the resolution parameter $\gamma$ which reduce the mean versatility, a measure of the uncertainty with which a node is assigned to a module (Shinn et al., 2017). The mean versatility does not provide an objective global optimum of the resolution parameter $\gamma$; instead, it serves to guide optimisation of $\gamma$ to local minima within neighbourhoods corresponding to the desired spatial resolution of the modules.

Five local minima of mean versatility in the range $0.01 < \gamma \leq 4.00$ were identified ($\gamma = 0.94, 1.06, 1.12, 1.19, 1.30$; Fig. 4.4A). Solutions of $\gamma < 0.85$ (where versatility $= 0$) yielded a trivial solution of a single module; local minima in the upper range of examined $\gamma$ values yielded too many modules to be practical (e.g.: 233 modules for $\gamma = 2.44$). The modular architecture corresponding to each local minimum of mean versatility was obtained as a consensus partition (Lancichinetti and Fortunato, 2012) across 1000 runs of the Louvain modularity algorithm using the corresponding value of $\gamma$ (Fig. 4.4B). For further analyses of the development of intra-and inter-modular structural network architecture, we chose the partition which divided the network into seven modules ($\gamma = 1.19$), for ease of comparison to the equally numerous cytoarchitectonic classes and functional networks.
Fig. 4.4 **Partitions of the age-invariant structural correlation network which minimise uncertainty of node affiliation to modules.** Module partitions were obtained for values of the resolution parameter corresponding to minima of mean versatility, a measure of the uncertainty with which a node is consistently affiliated to a module (Shinn et al., 2017). A) Mean versatility was calculated across 200 runs of the Louvain community algorithm for $\gamma = 0.01, 0.02, \ldots, 4.00$. Five local minima were selected. B) Community organisation for the five local minima indicated on panel A, using a consensus formulation of the Louvain algorithm (Lancichinetti and Fortunato, 2012) run 1000 times at each local minimum. Partition (iv) was chosen due to its 7-module solution, which facilitates comparison to the equally numerous cytoarchitectonic classes and functional networks.
The community partition consisted of seven modules, including three primary cortex modules: somatosensory (anterior parietal cortex), motor (posterior frontal cortex) and visual (occipital cortex), as well as an inferior-frontal/temporal module, a superior frontal module, a superior temporal/insular module and a parieto-occipital module. For details on this community structure and other modular partitions comprising different numbers of modules see Fig. 4.4. Analyses of changes in structural network architecture using other modular partitions (from the other local minima of $\gamma$) were qualitatively consistent (results not shown).

### 4.3.2 Age-resolved structural networks

To resolve age-related changes in structural networks, we used a “sliding window” analysis to estimate the structural correlation matrix separately for each of a series of subsets of the sample defined by overlapping age ranges or windows (Fig. 4.1B). The results of this analysis are naturally somewhat dependent on the sliding window parameters: the age-range spanned by each window and the incremental step between windows. Below we focus on results obtained with 9 windows of ~60 participants each, ranging from [14.1-16.0 years] to [22.0-25.0 years] with an incremental step of 30 participants (~1 year). In subsequent analyses, we explored a range of alternative sliding window parameters and demonstrated that our key results were robust to this methodological variation (see Chapter 5).

Globally, over the whole brain, there was a non-linear trend of reducing structural correlation from the youngest age window to the oldest age window (Fig. 4.5A). Relatively strong positive correlations at age 14 ($r > 0.31$) decreased sharply over the next few windows, with minimum mean correlation ($r \approx 0.22$) occurring at 19.59 years (95% confidence interval (CI) [19.37, 19.76] years) and then slightly increasing again towards age 24 ($\text{AIC}_{\text{spl}} < \text{AIC}_{\text{lin}}$, $r^2_{\text{adj}} = 0.52$, $P = 0.098$; Fig. 4.5Aii).

A potential drawback of the sliding window analysis is that it inevitably involves estimating inter-regional correlations on a subset of the sample ($N \approx 60$ per window), with commensurately reduced precision of estimation and therefore noisier graphs. We used a probabilistic threshold to control the edge-wise FDR at 1%, thus ensuring that the age-resolved graphs only included edges that were unlikely to represent false positive noise (Fig. 4.5B).

Focusing on the most statistically robust subset of edges (which passed the FDR threshold for significance), we found similar but clearer evidence for age-related global changes in structural organisation. The structural correlation distributions of the bootstrap-thresholded network became sparser over the course of adolescence (Fig. 4.5Ci). The edge density demonstrated a non-linear decrease ($\text{AIC}_{\text{spl}} < \text{AIC}_{\text{lin}}$) from 33.9% to a minimum of 8.2%
4.3 Results

Fig. 4.5 Global trajectories of age-resolved structural correlations and network connection density. A) Global trajectories of unthresholded structural correlations. (i) Development of the distribution of unthresholded correlations across age windows. Thin lines represent bootstrapped estimates, white lines represent the bootstrap mean. (ii) Changes in the average correlation. Black markers represent empirical data (error bars indicate the interquartile range across bootstraps), with corresponding regression line; the white marker indicates the trajectory minimum. Grey lines represent bootstrapped trajectories; the white dashed line represents the bootstrap mean. B) Each windowed matrix was thresholded using bootstrap. Within each window, 1000 sets of participants were resampled (with replacement) and used to construct correlation matrices. For each edge (correlation) within each window, the presence of a significant non-zero correlation (across bootstraps) was tested at the FDR-adjusted level of $\alpha_{FDR} = 0.01$. Consistent correlations were retained, while inconsistent correlations were assigned a value of 0. C) Global trajectories within thresholded structural correlation networks. (i) Development of the distribution of correlations retained after probabilistic thresholding across age windows. (ii) The number of edges retained after probabilistic thresholding, or edge density. The shaded area represents the 95% confidence interval of the spline fit.
at 19.45 years (95% CI [19.32, 19.59] years; $r^2_{adj} = 0.81$, $P = 0.0069$), which was similar in shape to the global trajectory of unthresholded correlation (Fig. 4.5Cii).

### 4.3.3 Regional development of age-resolved structural networks

Regional maturation of structural correlation networks was assessed by estimating the trajectories of changes in node degree, which is the number of correlations retained at each node (following bootstrap thresholding). Although there was regional heterogeneity in the trajectories of node degree (Fig. 4.6A), all regions that demonstrated significant evidence of non-zero change (linear or spline fit $P_{FDR} < 0.05$; 82 regions) followed a nonlinear trajectory ($AIC_{spl} < AIC_{lin}$), which for most regions (75/82) could be summarised by a younger phase (from 14 to 19 years approximately) of more-or-less rapid decrease in structural correlation followed by a levelling off or slight increase of structural correlation in an older phase (from 19 to 24 years approximately). This process could be summarised by two parameters: $D_{k_{max}}$, the difference between maximum and minimum degree; and $age(k_{min})$, the age at which node degree reached its minimum value (Fig. 4.6B).

Decreases in node degree were greatest in association cortical areas, such as bilateral dorsolateral prefrontal cortex, medial frontal cortex and supramarginal gyrus, as well as pre- and post-central gyri and several temporal cortical regions. Increases in node degree were less spatially clustered, occurring in isolated nodes within the right cingulate, superior frontal and parietal cortices as well as left cuneus (Fig. 4.6Ci). Association cortical areas also showed more prolonged decreases in structural correlation, reaching the minimum value of node degree later (Fig. 4.6Cii). Predictably, it follows that the extent of degree shrinkage $D_{k_{max}}$ was negatively correlated with the age at which degree reached its minimum value $age(k_{min})$, whether considering all regions (Spearman’s $\rho = -0.38$, $P_{Spearman} < 10^{-10}$, $P_{perm} < 10^{-5}$) or excluding regions whose minimum occurred at one of the limits of the age range (Spearman’s $\rho = -0.45$, $P_{Spearman} < 10^{-10}$, $P_{perm} = < 10^{-5}$; Fig. 4.6D).

### 4.3.4 Relationships of network development to thinning and myelination

To contextualise changes in structural network architecture with respect to maturation of cortical morphology, we related regional measures of cortical network development to rates of change of cortical thickness (CT) and magnetization transfer (MT, a measure of myelination), evaluated as the slope of a linear model fitted to the cross-sectional values. Following Whitaker et al. (2016), myelination analyses were conducted at 10 fractional depths between the pial surface and the grey/white matter boundary, as well as two absolute depths into
Fig. 4.6 Regional development of structural correlation networks. A) Cortical maps of node degree at five regularly sampled intervals of the developmental trajectories, showing a regionally heterogeneous decrease from young age. B) Definition of local measures of maturation, illustrated on a non-linearly decreasing trajectory (from the right dorso-lateral pre-frontal cortex). The maximum change in degree $\Delta k_{\text{max}}$ corresponds to the (absolute) difference (decrease or increase) in degree between the maximum and the minimum of the trajectory. The age at minimum degree $\text{age}(k_{\text{min}})$ corresponds to the timing of the minimum of the trajectory. C) Cortical maps of regional maturation measures for trajectories showing evidence of non-zero change (at $P_{\text{FDR}} < 0.05$), predominantly located in association cortex: (i) maximum change in degree, and (ii) age at minimum degree. D) Regions that show greater decreases in degree tend to reach minima of their trajectories later, whether considering all regions (grey) or excluding regions where the trajectory minimum occurs at extrema of the age range (black).
Fig. 4.7 Rates of thinning and myelination and derived results. A) Surface maps of rates of cortical (i) thinning and (ii) myelination (PU = percentage units). Only regions showing evidence of non-zero change (at $P_{FDR} < 0.05$) are shown. B) Rates of myelination across 308 regions as a function of cortical depth, including 10 fractional depths from the pial surface to the grey/white matter boundary (GM/WM), as well as two absolute depths into the white matter.

As already shown in (Whitaker et al., 2016), the greatest rates of thinning and myelination occur in association cortical areas (Fig. 4.7A), and the greatest rate of change of myelination occurs at 70% depth from the pial surface (Fig. 4.7B).

The maximum change in node degree was (weakly) positively correlated to the rate of thinning ($\Delta CT$; Spearman’s $\rho = 0.16$, $P_{\text{Spearman}} = 0.0050$, $P_{\text{perm}} = 0.023$; unaffected by excluding three outlier regions which showed $\Delta CT > 0$, Spearman’s $\rho = 0.15$, $P_{\text{Spearman}} = 0.0070$, $P_{\text{perm}} = 0.028$; Fig. 4.8Ai), and more strongly negatively correlated to the rate of intra-cortical myelination ($\Delta MT$; Spearman’s $\rho = -0.32$, $P_{\text{Spearman}} = 6.6 \times 10^{-9}$, $P_{\text{perm}} = 7 \times 10^{-4}$; Fig. 4.8Aii). The strength of association between local adolescent myelination (indexed by $\Delta MT$) and adolescent decrease of node degree (indexed by $\Delta k_{\text{max}}$) was greatest when $\Delta MT$ was measured at about 70% of cortical depth from the pial surface to the grey/white matter boundary (Fig. 4.8B).
4.3 Results

Fig. 4.8 *Relationship between maturation of cortical morphology and structural correlation networks*. A) Relationship between regional trajectories of cortical morphology and node degree. Maximum changes in nodal degree are only very weakly related to regional rates of i) thinning and ii) myelination (PU = percentage units). The direction of the relationships is such that cortical regions that myelinate more during adolescence are more likely to decrease in node degree and connection distance in the same period. B) Spearman correlation of the rate of change of myelination to maximal change in degree as a function of cortical depth, including 10 fractional depths from the pial surface to the grey/white matter boundary (GM/WM), as well as two absolute depths into the white matter.
Fig. 4.9 **Trajectories of Euclidean distance spanned by edges (retained after bootstrap-thresholding).** A) Global trajectory of average Euclidean distance. B) Definition of local measures of maturation, illustrated on a nonlinearly decreasing trajectory (from the right superior parietal cortex). The maximum change in distance $\Delta d_{\text{max}}$ corresponds to the (absolute) difference (decrease or increase) in degree between the maximum and the minimum of the trajectory. The age at minimum distance $\text{age}(d_{\text{min}})$ corresponds to the timing of the minimum of the trajectory. C) Cortical maps of properties of regional trajectories of average Euclidean distance: (i) maximum change in distance $\Delta d_{\text{max}}$, and (ii) age at minimum distance $\text{age}(d_{\text{min}})$. D) Regions that show greater decreases in distance tend to reach minima of their trajectories later, whether considering all regions (grey) or excluding regions where the trajectory minimum occurs at extrema of the age range (black). E) Regions that show greater decreases in degree also show greater decreases in nodal connection distance.

**4.3.5 Trajectories of Euclidean distance**

The global connection distance of the thresholded networks (the mean Euclidean distance subtended by bootstrap-thresholded edges) demonstrated a non-linear trajectory ($\text{AIC}_{\text{spl}} < \text{AIC}_{\text{lin}}$, $r_{\text{adj}}^2 = 0.67$, $P = 0.049$) characterised by a phase of relatively rapid decrease from 14 years to reach a minimum at about 18.7 years, followed by a phase of more stable connection distance (Fig. 4.9A).

Age-related non-linear changes in nodal connection distance (the mean Euclidean distance of all edges connecting a node within the bootstrap-thresholded network) were summarised using analogous parameters to node degree: $\Delta d_{\text{max}}$, the difference between max-
imum and minimum distance; and age($d_{\text{min}}$), the age at which nodal connection distance reached its minimum value. Nodes that demonstrated significantly reduced connection distance ($P_{\text{FDR}} < 0.05$) were located in left dorsolateral prefrontal cortex, left supramarginal gyrus and right superior parietal cortex (Fig. 4.9C). Decreases in node connection distance were negatively correlated with age at minimum connection distance, whether considering all nodes (Spearman’s $\rho = -0.38$, $P_{\text{Spearman}} < 10^{-10}$, $P_{\text{perm}} < 10^{-5}$) or excluding nodes whose minimum occurs at one of the limits of the age range (Spearman’s $\rho = -0.25$, $P_{\text{Spearman}} = 0.0027$, $P_{\text{perm}} = 0.0036$; Fig. 4.9D). Finally, decreases in node connection distance were positively correlated with decreases in node degree (Spearman’s $\rho = 0.32$, $P_{\text{Spearman}} = 1.9 \cdot 10^{-8}$, $P_{\text{perm}} < 10^{-5}$; Fig. 4.9E). In other words, nodes that had the greatest reduction in hubness during adolescence also tended to have the greatest reduction in connection distance.

### 4.3.6 Network changes in relation to the age-invariant network

Given that the most densely connected nodes (hubs) of the age-invariant structural correlation network are predominantly located in association cortex (Whitaker et al., 2016), which is also the location of greatest age-resolved decreases in structural correlation, it is not surprising that there is an inverse relationship between age-invariant node degree and maximum change in degree $\Delta k_{\text{max}}$, for both the weighted and binary age-invariant degree (Spearman’s $\rho$ respectively = -0.43 and -0.38, both $P_{\text{Spearman}} < 10^{-10}$, both $P_{\text{perm}} < 10^{-5}$). Node degree of the age-invariant network and age at minimum degree age($k_{\text{min}}$) were not strongly related.

We further studied adolescent changes in nodal topology in relation to the community structures of the human brain. Many community structures have been proposed to partition the cortex into a set of modules or sub-networks, each comprising a number of functionally and/or anatomically related cortical areas. Here we considered three complementary community structures: (i) the modular decomposition of the age-invariant structural correlation network (7 modules); (ii) the classic von Economo cytoarchitectonic partition of the cortex into 7 classes based on cortical lamination (von Economo and Koskinas, 1925); and (iii) the prior identification of 7 resting state networks derived from an independent resting state fMRI dataset using ICA (Yeo et al., 2011). The three classification systems had similar but not identical community structures; normalised mutual information (NMI, a measure of correspondence between two community structures) ranged from NMI = 0.39 for the relationship between the structural network modules and the resting state fMRI components to NMI = 0.29 for the relationships between both neuroimaging based community structures and the von Economo classification (Fig. 4.10A).
Adolescent tuning of association cortex in structural brain networks

Fig. 4.10 Adolescent development of structural networks in relation to human brain communities. (A) Comparison of the modular architecture of the age-invariant structural correlation network (middle) to two prior community structures – the von Economo atlas of cytoarchitectonic classes (von Economo and Koskinas, 1925; left) and seven functional intrinsic connectivity networks derived using independent fMRI data (Yeo et al., 2011; right). The alluvial diagrams between surface plots of community architecture indicate the amount of overlap between homologous community structures across templates. (B) Development of structural correlations within and between communities during adolescence. Left: maximum change in edge density \( \Delta D_{\text{max}} \) within and between all pairs of communities. Right: age at minimum edge density \( \Delta D_{\text{min}} \) within and between all pairs of communities. Dot markers indicate statistical significance of developmental change: small: \( P_{\text{FDR}} < 0.05 \), large: \( P_{\text{FDR}} < 0.01 \).
4.4 Discussion

References to color below correspond to Fig. 4.10. In the context of (i) the age-invariant structural network community structure, the greatest decreases in connection density $\Delta D_{\text{max}}$ were concentrated within the superior frontal module (blue) and within the superior temporal/insular module (purple); or between the superior frontal module and other modules (Fig. 4.10Biii). The age at minimum density age($D_{\text{min}}$) tends to be reached later within the same modules, as well as the occipito-parietal module (pink; Fig. 4.10Biv). In the context of (ii) cytoarchitectonic atlas of von Economo and Koskinas (1925), greatest decreases in edge density were concentrated within and between association cortical areas with lamination types 2 and 3 (described as granular isocortex; blue and green respectively) and particularly within class 3 (green; Fig. 4.10Bi). Association cortical trajectories tended also to reach the age of minimum edge density latest (Fig. 4.10Bii). In the context of (iii) fMRI resting state networks outlined by Yeo et al. (2011), the greatest decreases in edge density were concentrated within the fronto-parietal control network (orange) as well as between this network and the other networks (Fig. 4.10Bv). Minima of the trajectory are reached latest within the default mode network (salmon red) and the ventral attention network (pink), as well as between these two functional networks (Fig. 4.10Bvi). In summary, across the three community partitions, the greatest (and latest) decreases in connection density occurred within association cortical communities, and (to a lesser extent) between those association cortical communities and the remainder of the network.

4.4 Discussion

In the current study we set out to examine the developmental trajectories of human brain structural networks. To this end, we used a novel “sliding window” method of network analysis to resolve age-related changes in human brain structural correlations and probabilistically thresholded brain graphs estimated from MRI data on an age-stratified sample of healthy adolescents and young adults (N=297, aged 14-24 years). We found that global strength of structural correlation and the related topological property of edge density both decreased non-linearly as a function of age: an early phase (14-19.5 years approximately) of rapid decrease in structural correlation was followed by a later phase (20-24 years) of stable or slightly increasing structural correlation. At a regional or nodal level of analysis, cortical areas varied in the magnitude of age-related decrease in nodal degree $\Delta k_{\text{max}}$ and the age at which nodal degree reached its minimum value age($k_{\text{min}}$). The 75 cortical areas with significantly decreasing degree tended to mature later, i.e.: large negative $\Delta k_{\text{max}}$ was associated with older age($k_{\text{min}}$). Further, cortical areas with the greatest shrinkage of degree during adolescence also had the greatest shrinkage of connection distance, i.e.: large negative
$\Delta k_{\text{max}}$ was associated with large negative $\Delta d_{\text{max}}$. To contextualise these results, we showed that cortical areas with the greatest adolescent changes in brain structural connectivity were anatomically concentrated in regions of association cortex that had fast local rates of increasing intra-cortical myelination; and were topologically concentrated on the edges within frontal communities (von Economo classes 2 and 3 and the functional fronto-parietal control network) and the edges connecting frontal communities to the rest of the network. We propose that these results are consistent with the existence of a developmental window for tuning of association cortical connectivity by a combination of parsimoniously pruning some long distance connections while actively consolidating or myelinating the connections which survive.

### 4.4.1 MRI studies of adolescent structural brain network development

Adolescent changes in structural correlation networks have previously been investigated, as changes between neighbouring pairs of four discrete (non-overlapping) age-bins spanning the range 5-18 years (Zielinski et al., 2010; Khundrakpam et al., 2013). Zielinski et al. (2010) reported largely non-linear changes in the extent of seed-based structural correlation networks. Both the executive control network (seeded in the right dorsolateral prefrontal cortex) and the salience network (seeded in the right frontal insula), showed an increase in spatial extent, quantified as the number of voxels whose grey matter intensity significantly correlated with the seed. Conversely, our approach suggests a decrease in the structural correlation within association areas and related structural, cytoarchitectonic and functional communities. Beyond the difference in methods (voxel-wise seed-based vs. parcel-wise all-to-all regions), this discrepancy could be due to the different morphometric measures used, known to show differences in both trajectories of adolescent maturation (Wierenga et al., 2014; Ducharme et al., 2015), and (age-invariant) structural correlation (Sanabria-Diaz et al., 2010; Yang et al., 2016). Further, Khundrakpam et al. (2013) reported decreases in regional efficiency of primary sensorimotor regions, alongside increases in regional efficiency of paralimbic and association regions. These results align with our own, through the strong dependence of the properties of graphs thresholded to fixed edge densities (as in Khundrakpam et al. (2013)) on the mean of the correlation distributions from which they were derived. Networks with lower correlations lead to more random topology, exhibiting higher efficiency and lower clustering (Fornito et al., 2013; van den Heuvel et al., 2017). Therefore, our finding of decreases in structural correlation within association cortical areas aligns with reports by Khundrakpam et al. (2013) of increased regional efficiency in these regions. Beyond
development of structural networks resolved using distinct age-groups, several studies have investigated coordinated maturation of cortical morphology during adolescence (Raznahan et al., 2011; Alexander-Bloch et al., 2013b; Sotiras et al., 2017).

Adolescent development of structural connectivity has also been investigated using diffusion imaging and tractography, although such studies report heterogeneous findings. Lim et al. (2013) showed decreases in structural connectivity from childhood (4 years) to adulthood (40 years), concentrated predominantly on strong tracts, located within modules – which qualitatively agrees with our findings. However, Chen et al. (2013) reported increases in the number of streamlines and edge density from childhood (5 years) to adulthood (30 years). Recently, Baum et al. (2017) reported increases in within-module connectivity, and decreases in between-module connectivity in tractography-derived white matter networks. While tractography-derived structural connectomes show some overlap with structural correlation networks (Gong et al., 2012), interpretation of developmental changes in white-matter connectivity relative to development of structural correlations will require concurrent studies of both modalities in the same datasets. It is worth noting that when grey and white matter structural networks were both constructed using the same method (structural correlation), both showed similar patterns of correlation and similar developmental changes from 7 to 14 years (Moura et al., 2016).

Adolescent development of brain connectivity has also been investigated using fMRI. Early functional connectivity studies have reported increases in the strength of long-range and within-network functional connections, and decreases in the strength of short-range functional connections (Fair et al., 2009; Supekar et al., 2009; Dosenbach et al., 2010). Later studies have reported qualitatively similar findings, but with attenuated effect sizes following control for the effects of motion (Satterthwaite et al., 2012, 2013). While findings such as increasing within-module functional connectivity may seem to disagree with our findings of decreased within-network structural correlation, these constitute disparate modalities that have not always yielded concomitant results (Fornito and Bullmore, 2015). Beyond studies concurrently investigating adolescent development of structural and functional networks using the same dataset(s), the combination of structural, diffusion and functional MRI data using methods such as multimodal fusion (Calhoun and Sui, 2016), computational modelling (Breakspear, 2017) or morphometric similarity (Seidlitz et al., 2018) might be useful to reconcile findings from diverse modalities.
4.4.2 Relationship to axo-synaptic connectivity (and its adolescent pruning)

Our results extend previous studies of structural network development (Zielinski et al., 2010; Khundrakpam et al., 2013) by reporting smooth and non-linear trajectories of structural network development during adolescence. The early phase of major decrease in structural correlation, nodal degree, and nodal connection distance could represent loss of anatomical connectivity to association cortical areas. The simplest interpretation is that reduced structural correlation or degree represents pruning of synaptic connections or attenuation of axonal projections. There is a large body of prior evidence in support of the concept of synaptic pruning during adolescence (Huttenlocher and Dabholkar, 1997; Petanjek et al., 2011) and this mechanism has been suggested to explain age-related cortical shrinkage (Tau and Peterson, 2009), which was correlated with age-related degree shrinkage in these data. However, the security of this interpretation rests on the more fundamental assumption that structural correlation measured from MRI data on multiple subjects is a reasonable proxy marker of the average weight of axo-synaptic connectivity between regions (Alexander-Bloch et al., 2013a). Beyond humans (Gong et al., 2012), there is evidence of such correspondence from animal models (Yee et al., 2017).

The identification of structural correlation networks in mice (Pagani et al., 2016) suggests that they might encompass general features of cortical architecture. Specifically, up to 35% variance in structural correlation in mice was explained by a combination of tract-tracing-derived structural connectivity, gene expression and distance (Yee et al., 2017), providing a link of the macroscopic structural networks to underlying microscale cortical organisation. The relationship of structural correlation networks to gene expression has also been investigated within humans using the present data, demonstrating overlap between regional co-expression of genes (Hawrylycz et al., 2012), particularly of a subset of genes enriched in supra-granular layers of cerebral cortex, and structural correlation patterns (Romero-Garcia et al., 2017). Moreover, association cortical hubs of the (age-invariant) structural correlation network showed the greatest expression of genes related to synaptic transmission, oligodendroglia as well as schizophrenia, suggesting a potential pathogenic role in abnormal consolidation of association cortical regions (Whitaker et al., 2016). Generally, the profound adolescent maturational changes in cortical architecture are thought to underlie the frequent emergence of psychiatric disease in this period, as a result of abnormal development (Paus et al., 2008; Silbereis et al., 2016).
4.4 Discussion

4.4.3 Adolescent maturation of structural correlation and cortical structure

We note that the association of changes in structural network architecture to rates of cortical thinning is relatively weak. Given that (age-invariant) structural correlation networks are thought to emerge as a result of synchronised maturation (thinning) of cortical regions over adolescence (Raznahan et al., 2011; Alexander-Bloch et al., 2013b), perhaps the changes in structural correlation might be more closely related to changes in the rates of change of cortical thinning, which in a longitudinal dataset were shown to peak in adolescence (Zhou et al., 2015). An additional possible explanation for the adolescent decrease in structural correlation is a “decoherence” related to inter-individual differences in the timing of maturation of association areas – although the verification of such a hypothesis would again require longitudinal data. On a related note, recent work on functional connectivity has shown an adolescent increase in the “distinctiveness” of individual functional connectomes (Kaufmann et al., 2017).

Generally, the weakness of association between rates of change of morphology ($\Delta$CT and $\Delta$MT) and structural network architecture ($\Delta k_{\text{max}}$) suggests that rates of change of structural network properties explain substantial variation of brain structure with age, above and beyond the rates of thinning and myelination. As an intrinsic regional measure, cortical thickness can be considered less complex than a measure of relationships between regions (across participants) such as structural correlation; however, the biological hierarchy could well be the opposite, whereby cortical thickness and its changes might be a signature of underlying changes in axonal connectivity. This hypothesis could be tested, using invasive studies of concurrent development of axonal connectivity and cortical thickness in model species. In humans, the differential variance contained within cortical morphology and structural network architecture could be investigated through further within-population comparisons of these measures, in (i) their ability to discriminate between case-control populations, (ii) their association to behavioural and cognitive measures and (iii) their heritability. For example, patients with childhood-onset schizophrenia have shown differences in adolescent trajectories of both cortical thinning (Alexander-Bloch et al., 2014) and structural correlation (Zalesky and Breakspear, 2015) relative to healthy controls, but the measures have not been explicitly compared.

Notably, changes in structural network architecture were more strongly related to the rate of myelination (at 70% depth) than the rate of cortical thinning, suggesting that layer-specific intra-cortical myelination might be a more sensitive marker or cortico-cortical connectivity than cortical thickness (assuming, as above, that structural correlation is a marker
Adolescent tuning of association cortex in structural brain networks

of connectivity). Our finding of the strongest relationship between rate of myelination and amplitude of change in node degree being strongest at 70% depth between the pial surface and the grey/white matter boundary echoes our earlier identification of the myelination occurring fastest there (Whitaker et al., 2016). We have previously suggested a link of these changes to histological evidence of greatest rates of myelination at similar cortical depths in rodents (Mengler et al., 2014; Tomassy et al., 2014; Hammelrath et al., 2016).

4.4.4 Further considerations

Practical applicability of structural correlation networks is limited by the fact that they represent a group construct. Still, an advantage of structural correlation networks over structural connectomes derived from diffusion imaging using tractography is the relative simplicity of the structural MRI acquisitions compared to diffusion imaging, within which tractography presents considerable challenges (Thomas et al., 2014; Reveley et al., 2015; Maier-Hein et al., 2017). Efforts to derive fully individual networks from structural imaging (Tijms et al., 2012; Kong et al., 2014, 2015) including through the combination of multimodal features (Seidlitz et al., 2018) should increase the practical applicability of structural network research.

Further, in reporting a late maturation of association cortical regions, our results are potentially compatible with the developmental mismatch hypothesis, which proposes that late maturation of prefrontal regions (involved in cognitive control), compared to an earlier development of subcortical regions (implicated in reward processing) results in adolescent increases in risk-taking and sensation-seeking behaviours (Mills et al., 2014). However, the verification of such a hypothesis will require the inclusion of both subcortical regions and behavioural data in future analyses, as well as the use of a morphometric feature that - unlike CT - is defined in subcortex (e.g.: grey matter volume).

Finally, structural network architecture is known to mature across the lifespan (DuPre and Spreng, 2017), including during both early childhood (Geng et al., 2016) and late adulthood (Hafkemeijer et al., 2014). Our focused age-range prohibits us from conclusively ascertaining the specificity of these changes to adolescence. For example, extending the analyses presented herein to wider age-ranges would help disambiguate whether the non-linear decreases in structural correlation increase or level off in young adulthood.
CHAPTER 5

ROBUSTNESS OF SLIDING-WINDOW STRUCTURAL NETWORK ANALYSIS

5.1 Introduction

In this chapter, we test the robustness of sliding window analysis of structural brain networks, presented in Chapter 4, by conducting multiple ancillary studies. While we had no hypotheses about the shape of the maturational trajectories or the direction of the changes, the finding of a nonlinear decrease in structural correlation (and derived measures of edge density and degree), globally and locally, was somewhat surprising. This is one of the reasons why we conducted the following sensitivity analyses, to ensure that our findings are not caused or inflated by methodological choices or artefacts.

Given the dependence of the sliding window method on parameters, including the window width (number of participants included in each window) and the step size (number of participants differing between consecutive windows), we first explored a range of alternative sliding window parameters, to verify whether our key results are robust to this methodological variation.

Furthermore, depending on the window size, a relatively low number of summary data-points may be obtained, and fitting nonlinear trajectories to these may constitute overfitting. Thus, we explored whether our key results (reported in Chapter 4 using nine windows) would remain consistent when simple linear models are fitted instead of smoothing splines.

Moreover, to ascertain more detail about trajectories of structural correlation, we investigated components of Pearson’s correlation coefficient, including the covariance (numerator of the correlation coefficient) and the product of standard deviations (denominator of the correlation coefficient).

Subsequently, we inspected trajectories of structural network architecture for possible effects of gender.
Finally, we studied the effect of several potential artefacts, including the presence of regions with low reliability of structural correlations as well as irregularities in the age distribution of participants. Moreover, we investigated whether subtle non-linearities in trajectories of cortical thinning and myelination could be driving non-linearities in trajectories of structural correlation.

5.2 Methods

5.2.1 (In)dependence of results on sliding window parameters

We first verified that our results are qualitatively robust to the choice of sliding window parameters, by repeating our core analyses across a range of values of window width ({40, 60, 80} participants) and step size ({5, 10, 20} participants). The resulting number of windows $N_{\text{wind}}$ is calculated using the following equation:

$$N_{\text{wind}} = \left\lfloor \frac{N_{\text{part}} - \text{ww}}{ss} \right\rfloor$$  \hspace{1cm} (5.1)

where $N_{\text{part}}$ is the number of participants (here, 297), $\text{ww}$ and $ss$ are respectively the window width and step size (both a number of participants) and $[...]$ is the ceiling function, which rounds non-integer fractions up to the nearest integer (necessary for cases where due to the combination of parameters, one of the windows contains fewer participants than the rest). Trivially, the number of windows is inversely related to the window width and step size, such that greater windows and steps lead to fewer windows (and vice-versa). This affects the significance of model fits.

5.2.2 Linear rates of change of node degree

By fitting both linear and spline models to both trajectories, and comparing the quality of fit (given the parsimony of the model) using Akaike’s Information Criterion (AIC), we have shown that the trajectories of node degree exhibited by most regions are best described as nonlinear (Fig. 4.6). To estimate regional changes in degree, we have then used measures suitable for nonlinear models, including the maximum change in degree $\Delta k_{\text{max}}$ and the age at minimum degree age($k_{\text{min}}$). However, since the fitting of the nonlinear smoothing spline was conducted on few data-points, we wished to ascertain that results remain qualitatively consistent when the simplest possible model is used – the linear model, and the derived slope (the linear rate of change in degree $\Delta k_{\text{lin}}$).
5.2 Methods

5.2.3 Trajectories of structural correlation, covariance and variance

To ascertain more detail about trajectories of structural correlation, we investigated components of Pearson’s correlation coefficient \( \text{corr}(x,y) = \frac{\text{cov}(x,y)}{\sigma(x)\sigma(y)} \), including the covariance \( \text{cov}(x,y) \), numerator of the correlation coefficient) and the product of standard deviations \( \sigma(x)\sigma(y) \), denominator of the correlation coefficient). We estimated both the covariance and product of standard deviations across pairs of regional cortical thickness values for each sliding window, for both empirical data and across the 1000 bootstrapped trajectories. For each measure, we visualised shifts in the distribution of values, as well as the mean of the measure across the upper triangular part of the corresponding matrices.

5.2.4 Effects of gender

Further, we examined potential effects of gender by repeating sliding window analyses separately for each gender (149 female, 148 male participants). Consistently with analyses presented in Chapter 4, we used 9 windows - here containing ~30 participants each. Following estimation of global and nodal sliding window statistics separately for each gender within both unthresholded and bootstrap-thresholded networks (as described for all participants in Chapter 4), we fitted linear and spline models to the combined data, separately modelling effects of age, gender and the age-by-gender interaction.

Next, to ascertain the robustness of the obtained trajectories and to rule out potential underlying artefactual causes, we conducted a number of control experiments. These experiments, described in detail in sections 5.2.5-5.2.7 below, consisted in identifying sets of regions which could have been implicated in a particular artefact, and comparing trajectories of structural correlation averaged within and outside of these “masks”. We expected to see qualitatively similar changes with age in both resulting trajectories as evidence of the lack of influence of the potential underlying artefacts on the results.

5.2.5 Effects of regional reliability

First, we wished to verify whether estimation of structural correlations was more reliable in certain regions than others, and whether this may have affected results. Regional reliability was evaluated as the average similarity between regional correlation patterns generated from repeated random half-splits of the data. Specifically, we randomly subdivided subjects into two groups of equal size, built two structural correlation matrices by cross-correlating cortical thickness within each random half-group, and defined regional reliability as the correlation between the patterns of correlations (weights) of a region across the two splits
Robustness of sliding-window structural network analysis

(i.e.: correlations between rows – or equally, columns – of the resulting two matrices). Maps of reliability averaged over 1000 splits were thresholded using cross-correlations between random permutations of regions (the average reliability expected by chance), giving a set of “reliable” and “unreliable” regions. These two sets were used as masks, within which both empirical and bootstrapped correlations were averaged, to evaluate the effects of regional reliability on the developmental trajectories of structural correlations.

5.2.6 Effects of irregularities in age-distribution of participants

Although the NSPN study was carefully designed to sample a uniform distribution of participants over age, there remain unavoidable small irregularities in age difference between consecutive participants (seen in the plot of participant age as a function of participant rank, Fig. 5.5A; a perfectly uniform distribution of participants across age would lead to a straight line). To evaluate the potential effect of these irregularities on results, we calculated age differences between consecutive participants and averaged them within age-bins. We then cross-correlated the resulting time-course with regional time-courses of node strength (as a function of age) explained by local non-uniformities in the age distribution of participants. Further, we divided regions into two groups – those “affected” regions for which age-difference-fluctuations explained more than 10% variance (over time), and the remaining “unaffected” regions. We used these regions as masks, within which both empirical and bootstrapped correlations were averaged. Finally, we compared effect sizes and nonlinearity of the resulting average trajectories.

5.2.7 Effects of non-linearities in trajectories of thinning and myelination

Although trajectories of cortical thinning (and myelination) have been reported as linear in the current (cross-sectional) dataset (Whitaker et al., 2016), subtle non-linearities may be present in the data, driving the nonlinear trajectories of structural correlation (and derived measures of edge density and node degree). To rule out effects of subtle non-linearities in trajectories of cortical thinning (and myelination) on trajectories of structural correlation, we fitted (potentially non-linear) smoothing splines to trajectories of both cortical thickness and myelination at all 308 regions, using data from all 297 participants included in this study. We used the same smoothing spline models as for structural network trajectories (see Chapter 4), constraining the trajectories to be approximately as smooth as quadratic models (i.e.: \( df \leq 3.5 \)). We then compared the quality of linear and smoothing spline fits, given the
Results

5.3 Results

5.3.1 (In)dependence of results on sliding window parameters

We first evaluated robustness of our findings to parameters of the sliding window method, varying the window width and step size over ranges of \{40, 60, 80\} and \{5, 10, 20\} participants respectively. The results confirm the robustness of our main findings (Table 5.1 and Fig. 5.1).

The exact quantitative estimates of effect sizes and significance of fits vary across windows. Specifically, the greatest variation can be seen in the numbers of regions showing significant change with age (Fig. 5.1 A-C iii,iv) – the number of regions showing significant changes increases with the number of windows. Furthermore, the range of edge densities within each trajectory varies across parameters, although the shape of the trajectory remains qualitatively consistent (Fig. 5.1 A-C ii). Finally, smaller steps between windows lead to local fluctuations in correlation, likely linked to fluctuations in the local edge density of participants (Fig. 5.1 Ai). However, this is somewhat mitigated by the probabilistic bootstrap-based thresholding, which eliminates inconsistent edges (Fig. 5.1 Aii). We note that our choice of parameters for the main analyses (presented in Chapter 4) was designed to minimise overlap of neighbouring windows and maximise the conservation of covariates across windows.

Sub-sample analysis

Finally, to further investigate potential effects of non-linearities in trajectories of cortical thinning on nonlinear trajectories of structural correlation, we fitted linear models separately to the youngest and oldest halves of our participants (respectively 149 participants (75 female), aged 14.10-18.63 years, and 148 participants (74 female) aged 18.64-24.98 years), and quantified the difference in rates of change between the two. We then compared the cortical distributions of these differences to the cortical distributions of our nonlinear trajectories of structural network architecture, to ascertain if there is any overlap between the two.
Robustness of sliding-window structural network analysis

### Table 5.1 (In)dependence of results on sliding window parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>µ</th>
<th>r² adj (P)</th>
<th>Degree Nonlin.</th>
<th>Degree Lin.</th>
<th>DCT vs Dk max</th>
<th>DMT vs Dk max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Window width</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>20</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Results from our main analyses, across main parameters reported in Chapter 4 (top row) and all combinations of window width and step sizes of {40, 60, 80} and {5, 10, 20} participants. From left to right, the table lists the number of windows resulting from each combination of parameters, effect sizes and P values for the mean (unthresholded) correlation and (thresholded) edge density; the number of regions showing evidence of nonlinear increases or decreases in node degree (at P_{FDR} < 0.05); as well as effect sizes and P values of the relationship of maximum change in node degree to the rates of thinning and myelination.
5.3 Results

Fig. 5.1 (In)dependence of results on sliding window parameters. Results for three selected combinations of parameters, including A) window width of 80 participants with a step size of 5 participants, B) window width of 60 participants with a step size of 10 participants and C) window width of 40 participants with a step size of 20 participants. Results shown are: (i) development of mean correlation (in unthresholded networks), (ii) development of edge density (in bootstrap thresholded networks), and local measures of (iii) maximum change in degree $\Delta k_{\text{max}}$ and (iv) age at minimum degree age($k_{\text{min}}$). Cortical effect sizes are visualised only for regions whose trajectories satisfy $P_{\text{FDR}} < 0.05$. 
5.3.2 Linear rates of change of node degree

To ensure that we did not overfit the data by fitting smoothing splines to a small number of data points, we inspected the cortical distribution of regional changes in network architecture resulting from the fitting of simpler linear trajectories. Rates of linear change in node degree $\Delta k_{\text{lin}}$ demonstrated greatest decreases in degree in association cortical areas (Fig. 5.2A), consistently with the nonlinear measure of maximum change in node degree $\Delta k_{\text{max}}$. Further, linear rates of change of degree showed consistent direction and magnitude of correlation to rates of both cortical thinning ($\Delta CT$; $\rho = 0.20$, $P_{\text{Spearman}} = 0.00048$, $P_{\text{perm}} = 0.037$; Fig. 5.2Bi) and myelination ($\Delta MT$; $\rho = -0.33$, $P_{\text{Spearman}} = 5.4 \times 10^{-9}$, $P_{\text{perm}} = 0.0023$; Fig. 5.2Bii).

5.3.3 Trajectories of structural correlation, covariance and variance

To "decompose" trajectories of structural correlations into constituent elements, we visualised global maturational trajectories of its numerator and denominator - respectively the covariance and product of standard deviations. We found that trajectories of mean covariance and product of standard deviations were qualitatively consistent with the trajectory of mean correlation, exhibiting a nonlinear decrease followed by a slow linear increase (covariance: $\text{AIC}_{\text{spl}} < \text{AIC}_{\text{lin}}$, $r^2_{\text{adj}} = 0.61$, $P = 0.059$, age$_{\text{min}} = 19.9$ y; Fig. 5.3Bii; product of standard deviations: $\text{AIC}_{\text{spl}} < \text{AIC}_{\text{lin}}$, $r^2_{\text{adj}} = 0.67$, $P = 0.035$, age$_{\text{min}} = 20.7$ y, Fig. 5.3Cii).
Fig. 5.3 **Decomposition of structural correlation into structural covariance and the product of standard deviations.** Changes in the A) correlation, B) covariance and C) product of standard deviations of cortical thickness as both (i) distributions and (ii) means as a function of age. In panels (ii), black markers correspond to empirical data and spline fits, while grey lines correspond to trajectories of the bootstrapped correlations (with the white dashed line as their mean); vertical black lines correspond to inter-quartile ranges across bootstraps within each age-bin.
5.3.4 Effects of gender

We next revisited global and regional analyses while paying attention to potential effects of gender, or an age-by-gender interaction. Analysis of gender differences failed to show effects of gender or an age-by-gender interaction at the global level, within the mean correlation ($P_{\text{gender}} = 0.49$, $P_{\text{inter}} = 0.97$) or within the edge density of the thresholded networks ($P_{\text{gender}} = 0.54$, $P_{\text{inter}} = 0.94$). At the nodal level, after FDR adjustment for multiple comparisons ($\alpha_{\text{FDR}} = 0.05$), 4 regions showed an effect of gender, and 16 regions showed evidence of an age-by-gender interaction. However, due to the lack of spatial clustering of these results, and inconsistency of the gender-specific trajectories across regions, these results are not compelling - suggesting a lack of substantial evidence for gender-related effects in these data.

5.3.5 Effects of regional reliability

Subsequently, we investigated the impact of a number of potential underlying artifacts on our results. We first focused on the reliability of regional structural correlations, by comparing regional correlation patterns between 1000 random half-splits of the full dataset. Results show a pattern of reliable regions located predominantly in superior frontal cortex and postcentral gyrus (Fig. 5.4A). When regional reliability was re-estimated within sliding-window bins, its average value across bins was highly correlated to the overall reliability (Fig. 5.4B). This, together with weak evidence of changes in structural correlation reliability ($r^2_{\text{adj}} = 0.12$, $P = 0.51$; Fig. 5.4C), justifies the use of the overall estimate of regional reliability (Fig. 5.4Aii) as a mask to distinguish "reliable" and "unreliable" regions.

The trajectory of average structural correlations within the "reliable" regions shows both greater evidence of change as well as a more nonlinear trajectory ($r^2_{\text{adj}} = 0.81$, $P = 0.0073$, $df = 3.3$; Fig. 5.4D) than the trajectory of change within the remaining "unreliable" regions ($r^2_{\text{spl}} = 0.31$, $P = 0.27$, $df = 3.1$; Fig. 5.4E) (Wilcoxon Rank-sum test of difference in $df$ across bootstraps: $P < 10^{-10}$). We thus conclude that the nonlinear decrease in structural correlation isn’t driven by a potential unreliability of structural correlation estimates; if anything, it might be attenuated by certain less reliable regions.

5.3.6 Effects of irregularities in age-distribution of participants

We next inspected whether irregularities in the inevitably non-uniform age distribution of our participant sample could artefactually influence the construction of age-resolved structural networks using sliding windows. To do so, we averaged age-gaps between consecutive
Fig. 5.4 Effects of regional reliability on trajectories of structural correlations. A) (i) Reliability of regional cortical thickness correlations, estimated as the average correlation between rows of pairs of correlation matrices generated from 1000 random half-splits of all 297 participants. (ii) “Reliable” regions, thresholded using random permutation of regions (rows) across the random half-splits (104/308 regions = 33.8%). B) When re-estimated within each (sliding) window, the average reliability is highly correlated to the overall (static) reliability. This, together with weak evidence of change in regional reliability across windows (C), justifies using reliable regions (Aii) as a mask to investigate the effect of regional reliability on structural correlations. D) The mean correlation within reliable regions is higher, shows a greater magnitude of change and a more nonlinear trajectory than E) the mean correlation within the remaining regions.
participants (Fig. 5.5B), and correlated the resulting pattern with regional age-related fluctuations in node strength. The regions whose strength fluctuates in relation to irregularities in the age-distribution of participants (as evidenced by a high correlation between the two patterns) are not spatially clustered, nor are they prominently located in association cortex - where the greatest decreases in correlation strength and node degree were found (Fig. 5.5Ci). For a few regions, the proportion of variance in strength explained by inhomogeneities in the age distribution was surprisingly high (r² up to ~0.8), although most regions showed much weaker relationships to the artefactual pattern of age inhomogeneities (Fig. 5.5Cii).

To investigate whether regional trajectories of structural correlation show a dependence on the extent of regional alignment with fluctuations in age inhomogeneities, we divided regions into two groups: "affected" regions for which age-difference fluctuations explained more than 10% variance over time (102/308 = 33.1% regions), and the remaining "unaffected" regions (206/308 = 66.9% regions). Following averaging of structural correlation trajectories within these subsets of regions, regions that are only weakly affected by fluctuations in the age-distribution of participants show greater evidence for change as a function of age as well as more nonlinear trajectories (r²_adj = 0.61, P = 0.064, df = 3.26; Fig. 5.5D) than regions which are more strongly affected by these fluctuations (r²_adj = 0.33, P = 0.22, df = 2.95; Fig. 5.5E) (Wilcoxon Rank-sum test of difference in df across bootstraps: P < 10⁻¹⁰). Therefore, we conclude that the nonlinear age-related decrease in structural correlation is unlikely to be driven by potential artefacts related to small irregularities in the age-distribution of participants; if anything, it might be attenuated by this.

5.3.7 Effects of non-linearities in trajectories of thinning and myelination

Subsequently, we wished to rule out the potential effects of subtle non-linearities in trajectories of cortical thinning and myelination on trajectories of structural correlation. Thus, we fitted smoothing splines as a function of age to regional values of CT and MT across all 297 participants, and inspected the nonlinearity of the resulting trajectories using the AIC of the splines (AIC_{spl}) relative to linear models fitted to the same data (AIC_{lin}), as well as the number of degrees of freedom (df) of the spline fit. A trajectory with df = 2 is linear - the two degrees of freedom in this case correspond to the intercept and slope; as the trajectory becomes more non-linear, its number of degrees of freedom increases.
5.3 Results

Fig. 5.5 Effects of inhomogeneity in the age-distribution of participants. A) While participants are sampled relatively uniformly, there remain inevitable small inhomogeneities in the age-gaps between consecutive participants (a perfectly homogenous distribution of participants with age would result in a straight line). Vertical dashed lines correspond to boundaries of the five age-strata of the NSPN study, within which participants were recruited. B) There is a difference in average gaps between consecutive participants across the age-range, such that later windows show a greater gap between consecutive participants. C) Cross-correlating the pattern of age-gaps between consecutive participants with nodal strength yields several regions within which a high proportion of variance in strength with age appears to be caused by the age-inhomogeneities. (ii) However, most regions are only weakly correlated to this pattern. D) Averaging structural correlations within regions whose pattern of node strength fluctuations is relatively unaffected by age inhomogeneities (arbitrary cut-off at $r^2(str, \Delta_{age}) < 0.1$; 206/308 regions = 66.9%) leads to more non-linear trajectories that present greater magnitudes of change than E) averaging structural correlations within regions whose pattern of node strength fluctuations is more strongly affected by age inhomogeneities (arbitrary cut-off at $r^2(str, \Delta_{age}) > 0.1$; 102/308 regions = 33.1%).
Nonlinearities in cortical thickness

Within cortical thickness data, values of AIC were in general very similar for the linear model and smoothing splines. Although 203/308 regions (65.9%) displayed a better fit with the smoothing spline (\(\text{AIC}_{\text{spl}} < \text{AIC}_{\text{lin}}\)), most of these trajectories remained quasi-linear – the distribution of degrees of freedom remained heavily skewed towards 2 (Fig. 5.6A), with only 112/308 regions (36.4%) displaying weak evidence of nonlinearity (\(df_{\text{CT}} > 2.01\); Fig. 5.6B). Inspecting examples of the 10 "most linear" and 10 "most non-linear" trajectories (Fig. 5.6 C,D) indicates heterogeneity in the non-linearities, with few trajectories showing a similar shape of trajectory to structural correlation. Subsequently, averaging structural correlations within the 196/308 regions (63.6%) showing linear thinning (\(df_{\text{CT}} = 2\); grey in Fig. 5.6B) led to similarly nonlinear trajectories (\(r^2_{\text{adj}} = 0.53, P = 0.099, df = 2.76\); Fig. 5.6E) as averaging structural correlations within the remaining 112/308 (36.4%) "non-linear" regions (\(r^2_{\text{adj}} = 0.55, P = 0.083, df = 2.76\); Fig. 5.6F). In fact, across bootstraps, trajectories of structural correlation within regions showing subtle non-linearities in cortical thinning had lower median \(df\) (= 2.82) than regions showing purely linear trajectories of thinning (median \(df = 3.03\); Wilcoxon rank-sum test \(P = 1.29 \cdot 10^{-5}\)).

Additionally, we verified that the degrees of freedom of cortical thickness trajectories \(df_{\text{CT}}\) are not spatially correlated to: the degrees of freedom of trajectories of nodal correlation (Spearman \(\rho = 0.024, P_{\text{Spearman}} = 0.68, P_{\text{perm}} = 0.26\)), the degrees of freedom of trajectories of node degree (Spearman \(\rho = 0.054, P_{\text{Spearman}} = 0.34, P_{\text{perm}} = 0.11\)), the maximum change in degree \(\Delta k_{\text{max}}\) (Spearman \(\rho = -0.072, P_{\text{Spearman}} = 0.20, P_{\text{perm}} = 0.11\)) or the age at minimum degree \(k_{\text{min}}\) (Spearman \(\rho = 0.0012, P_{\text{Spearman}} = 0.98, P_{\text{perm}} = 0.57\)).

Taken together, these results indicate that subtle non-linearities in trajectories of cortical thinning are unlikely to be driving our main finding, of a nonlinear trajectory of structural correlation.

Nonlinearities in myelination

Further, we repeated the above analyses, inspecting subtle non-linearities in trajectories of cortical myelination. As in the main analyses and as in Whitaker et al. (2016), we focused on myelination estimated at approximately 70% depth between the pial surface and the grey-white matter boundary.

For trajectories of myelination, values of AIC were again very similar for the linear model and smoothing splines. Although 236/308 regions (76.6%) displayed a better fit with the smoothing spline (\(\text{AIC}_{\text{spl}} < \text{AIC}_{\text{lin}}\)), most of these trajectories remained quasi-linear – the distribution of degrees of freedom remained heavily skewed towards 2 (Fig. 5.7A), with only
Fig. 5.6 Effects of regional reliability on trajectories of structural correlations. A) The distribution of nodal degrees of freedom for spline trajectories of cortical thinning. Most regions show linear trajectories, with \( df_{CT} = 2 \). B) Cortical surface plots for nodal degrees of freedom, for the subset of regions showing subtle non-linearities \( (df_{CT} > 2.01) \); regions showing linear thinning \( (df_{CT} = 2) \) are plotted in gray. E) Averaging structural correlations within regions showing purely linear trajectories \( (df_{CT} = 2; 196/308 \text{ regions} = 63.6\%) \) leads to equally non-linear trajectories (of structural correlation) as averaging structural correlations within regions showing subtle non-linearities \( (df_{CT} > 2.01; 112/308 \text{ regions} = 36.4\%). \)
Fig. 5.7 **Effects of subtle non-linearities in myelination trajectories.** A) The distribution of nodal degrees of freedom for spline trajectories of cortical myelination. Most regions show linear trajectories, with $df_{MT} = 2$. B) Cortical surface plots for nodal degrees of freedom, for the subset of regions showing subtle non-linearities ($df_{MT} > 2.01$); regions showing linear thinning ($df_{MT} = 2$) are plotted in gray. E) Averaging structural correlations within regions showing purely linear trajectories ($df_{MT} = 2$; 196/308 regions = 63.6%) leads to equally non-linear trajectories (of structural correlation) as averaging structural correlations within regions showing subtle non-linearities ($df_{MT} > 2.01$; 112/308 regions = 36.4%).
115/308 regions (37.3%) displaying weak evidence of nonlinearity ($df_{MT} > 2.01$; Fig. 5.7B). Inspecting examples of the 10 “most linear” and 10 “most non-linear” trajectories (Fig. 5.7 C,D) indicates greater homogeneity in the non-linearities than trajectories of cortical thickness; still, the shape of this trajectory does not resemble trajectories of structural correlation. Subsequently, averaging structural correlation within the 193/308 regions showing linear myelination ($df_{MT} = 2$; grey in Fig. 5.7B) led to similarly nonlinear trajectories ($r^2_{adj} = 0.58$, $P = 0.075$, $df = 2.76$; Fig. 5.7E) as averaging structural correlation within the remaining 115/308 (37.3.6%) “non-linear” regions ($r^2_{adj} = 0.51$, $P = 0.083$, $df = 2.76$; Fig. 5.7F). Interestingly, across bootstraps, trajectories of structural correlation within regions showing subtle non-linearities in cortical myelination had higher median $df$ (= 3.00) than regions showing purely linear trajectories of myelination (median $df = 2.88$; Wilcoxon rank-sum test $P = 0.036$).

Additionally, we inspected whether degrees of freedom of myelination trajectories $df_{MT}$ correlate with measures derived from (nonlinear) trajectories of structural correlation. The correlations were weak for the degrees of freedom of trajectories of nodal correlation (Spearman $\rho = -0.013$, $P_{\text{Spearman}} = 0.82$, $P_{\text{perm}} = 0.37$), the degrees of freedom of trajectories of node degree (Spearman $\rho = 0.024$, $P_{\text{Spearman}} = 0.68$, $P_{\text{perm}} = 0.47$) and the maximum change in degree $\Delta k_{max}$ (Spearman $\rho = 0.015$, $P_{\text{Spearman}} = 0.79$, $P_{\text{perm}} = 0.40$) but stronger for the age at minimum degree age($k_{min}$) (Spearman $\rho = -0.14$, $P_{\text{Spearman}} = 0.016$, $P_{\text{perm}} = 0.059$).

Taken together, these findings indicate that subtle non-linearities in trajectories of cortical myelination may have a stronger influence on structural correlation than subtle non-linearities in trajectories of cortical thinning. The idea that myelination is a stronger driver of structural correlation than cortical thinning would also be supported by our finding (reported in Chapter 4) that the maximum change in degree $\Delta k_{max}$ is more strongly correlated to the rate of cortical myelination ($D_{MT}$) than thinning ($D_{CT}$).

**Sub-sample analysis**

Finally, to comprehensively inspect CT and MT data for possible non-linearities which might be driving the non-linearly decreasing trajectory of structural correlation, we fitted linear models separately to two age-halves of the sample (respectively 149 participants aged 14.10-18.63 years, and 148 participants aged 18.64-24.98 years). We found that the rate of cortical thinning is generally slower in the first (younger) half of the sample (Fig. 5.8Ai) than in the second (older) half (Fig. 5.8Aii), with the “acceleration of thinning” occurring fastest in association cortex (Fig. 5.8B). These results agree with a previous longitudinal study, showing an acceleration of cortical thinning in adolescence (Zhou et al., 2015). Conversely, rates of myelination are faster in the first (younger) half-
Robustness of sliding-window structural network analysis

Fig. 5.8 Rates of thinning and myelination in sub-samples of participants. Top row: A) The rate of thinning in the (i) first and (ii) second half-samples of participants, and B) the difference between the two. Bottom row: C) The rate of myelination in the (i) first and (ii) second half-samples of participants, and D) the difference between the two.

Sample (Fig. 5.8Ci) than in the second (older) half (Fig. 5.8Cii), although in this case the difference is greatest in superior parietal regions (Fig. 5.8D).

The location of the greatest differences in rates of thinning (Fig. 5.8B) and myelination (Fig. 5.8D) between the two half-samples align with the locations of greatest non-linearities in these trajectories (respectively Fig. 5.6B and Fig. 5.7B); while the trajectory of cortical thinning demonstrates a faster rate of change in the second half of the age-range, the trajectory of myelination shows a faster rate of change in the first half of the age range. The differences in slope in Fig. 5.8 B and D are both negative due to the signs of the trajectories; in the case of thinning (where both slopes are negative) this corresponds to a faster decrease in thickness in the second half of the age range (Fig. 5.8B), whereas in the case of myelination (where both slopes are positive) this corresponds to a slower increase in myelination in the second half of the age range than in the first (Fig. 5.8D).

To summarise, the differences in the rates of thinning between the two half samples differ from the trajectory of structural correlation, which shows a faster rate of change (decrease) in the first half of our age-range, than in the second half (where it levels off or even slightly increases). The fact that myelination is faster in the first half than in the second shows closer alignment with trajectories of structural correlation; however, the loci of these effects
on the cortex differ. Thus, this analysis provides further evidence that the non-linearity within trajectories of structural correlation is likely not driven by subtle non-linearities within trajectories of cortical thinning and myelination – although it appears more strongly related to the latter than to the former.

5.4 Discussion

In this chapter we investigated the robustness of sliding window construction of structural networks, including the dependence of results on input parameters as well as various properties of the underlying data, such as inhomogeneities in the age distribution of participants or non-linearities in developmental trajectories of the data used to construct the networks.

5.4.1 Parameters of the sliding window method

The estimated changes in structural network organisation are inevitably dependent on parameters of the sliding window method used. The selection of sliding window parameters, including window width and step size (in units of number of participants) involves several trade-offs. On one hand, selecting a wider window increases the robustness of correlations within each of those windows, as they are estimated using more participants; on the other hand, the median ages of participants within each window will cover a narrower portion of the overall age-range. Furthermore, while a smaller step size will provide a greater density of windows and hence time-points for curve fitting and trajectory characterisation, a denser sampling of data will exacerbate issues with the inevitably uneven distribution of subjects across the age-range studied, which in effect corresponds to an unevenly sampled time series (Eckner, 2014). Specifically, while decreasing the step-size leads the method further from a discrete and closer to a continuous characterisation of the data, failure to take into account the uneven sampling may introduce issues such as artefactual fluctuations in structural correlation related to local fluctuations in participant density. Future development of tools for the analysis of unevenly sampled time series should help alleviate these issues. Furthermore, with a denser sampling of participants, covariates might not be well conserved across windows, although this can potentially be controlled for using regression. Finally, with a smaller step size, the dependence between consecutive datapoints should technically be taken into account when quantitatively estimating effect sizes and P values for the smooth trajectories of structural network properties.

Moreover, depending on the combination of sliding window parameters used, relatively few summary data points may be obtained. The subsequent fitting of nonlinear smoothing
splines (with up to ~3.5 degrees of freedom) to such scarce data warrants care when interpreting evidence of non-linearity – despite evidence from the AIC (as well as BIC) that smoothing splines provide a better quality of fit than linear models. Still, it is reassuring that trajectories remain consistently nonlinear across bootstrapped samples (within unthresholded correlation networks) and that evidence of a nonlinear trajectory seems more pronounced after thresholding.

Finally, although technically not a parameter of the sliding window method itself, the choice of model fitted to the summary window datapoints is a key decision of the age-resolved network analysis pipeline. In particular, the scarcity of summary datapoints may lead to overfitting, as well as to uncertainties in measures used to characterise the maturational trajectories - including the measures of maximum change and age at minimum of the trajectory. Thus, it is reassuring that changes in structural network architecture remain qualitatively consistent in both their spatial distribution and relationship to changes in morphology when simple linear models are used.

5.4.2 Properties of data used to construct the age-resolved network

We investigated the effect of several potential artefacts, including the presence of regions with low reliability of structural correlations. Notably, we found a high reliability of structural correlations in similar locations to regions reported as most reliable during estimation of cortical thickness (Iscan et al., 2015; Liem et al., 2015; Madan and Kensinger, 2017). One exception is the high reliability of structural correlations in inferior temporal cortex in our data, which generally shows low reliability of CT estimates; this is likely due to the manual correction of our data in these known “dropout” locations. The reliability of structural correlation estimates remains a topic for further study.

A further potential underlying artefact are inhomogeneities in the age distribution of participants - which, as mentioned above, corresponds to an unevenly sampled time series. It should be noted that in data as complex as MRI-derived estimates of cortical thickness and myelination, such artefacts can most likely not be controlled for using interpolation, which is the simplest method for dealing with unevenly distributed data (Eckner, 2014).

Finally, we investigated whether subtle non-linearities in trajectories of cortical thinning and myelination could be driving non-linearities in trajectories of structural correlation. Although neither non-linear CT or MT effects are especially strong, subtle non-linearities in trajectories of cortical myelination appear somewhat more related to structural correlation trajectories than subtle non-linearities in trajectories of cortical thinning.
Still, taken together, we found no substantial evidence that the effect of the above artefacts could account for our main finding of a non-linear age-related decrease in structural correlations.

5.4.3 Methodological considerations

Recently, a number of studies have pointed out effects of participant motion on the quality of structural MRI scans, including on estimates of regional morphological measures such as cortical thickness (Reuter et al., 2015; Alexander-Bloch et al., 2016; Savalia et al., 2017). While we have carried out stringent quality control of our structural scans and FreeSurfer reconstructions of cortical thickness (details in section 4.2.2 in Chapter 4), we cannot completely rule out potential artefactual effects of motion on our results. Thus, further analysis of structural correlation development in datasets including estimates of head motion from volumetric tracking (Tisdall et al., 2012, 2016) or novel automated estimates of data quality (Shehzad et al., 2015; Pizarro et al., 2016; Rosen et al., 2017) will be important in the future.

Further, it remains ambiguous whether the tendency of the global trajectory of structural correlation to slightly increase from the minimum around age 19 towards age 24 years is significant, or whether the trajectory can be seen as levelling-off. It seems reasonable that the few nodes presenting increases in structural correlation (e.g.: within right cingulate cortex) would be driving this effect. Thus, until these results are validated in an additional dataset, care is necessary in some aspects of their interpretation.

5.4.4 Conclusion

This section of the thesis focused on a method for the construction of age-resolved structural correlation networks. During adolescence, these networks demonstrate a non-linear reduction of connectivity of association cortical areas, predominantly in frontal cortex, that is compatible with a developmental process of pruning combined with consolidation of surviving connections. These results are robust to variations in the age-resolved network construction and analysis pipeline, and do not appear to be driven by a number of potential underlying artefacts.
PART III

ANALYSIS OF UNTHRESHOLDED FUNCTIONAL NETWORKS
ADOLESCENT DEVELOPMENT OF FUNCTIONAL BRAIN NETWORKS

6.1 Introduction

As seen in Part II of this thesis, the human brain undergoes substantial changes in structure over adolescence. Maturation of intrinsic regional properties such as cortical thickness or myelination (Miller et al., 2012; Wierenga et al., 2014; Whitaker et al., 2016) underlies the development of distributed structural networks (Hagmann et al., 2010; Zielinski et al., 2010; Khundrakpam et al., 2013). These changes are complemented by the parallel development of functional connectivity, commonly defined as the inter-dependence of activity amongst distributed sets of regions (Friston, 1994; Bullmore and Sporns, 2009) and hypothesised to subserve communication (Fries, 2005).

Numerous studies have described adolescent development of functional network organisation using fMRI, a large proportion of which have applied graph theoretical methods (Bullmore and Sporns, 2009; Fornito et al., 2016). Initial studies of resting-state functional connectivity (FC) development have converged on an increase in the strength of long-range connections accompanied by a decrease in the strength of short-range connections (Fair et al., 2007, 2009; Supekar et al., 2009; Dosenbach et al., 2010), hypothesised to capture a maturational shift from a local to a distributed organisation (Fair et al., 2009). However, these early studies were conducted before the realisation that regression of motion parameters and their derivatives from BOLD time series (Friston et al., 1996), the prevalent method for motion-correction of fMRI data at the time, is insufficient to correct for the confounding effects of in-scanner motion (Power et al., 2012; Satterthwaite et al., 2012). Effects of motion on functional connectivity estimates are particularly problematic in this context as (i) the magnitude of in-scanner motion tends to decrease with age between childhood and adulthood (i.e.: younger children tend to move more; Satterthwaite et al., 2013), and (ii) motion is
known to differentially affect short and long-distance edges (i.e.: motion tends to increase correlation between spatially proximal nodes, and decrease correlation between distal nodes; Power et al., 2012). Thus, a study on the impact of motion on developmental trajectories of FC reported similar findings as the aforementioned prior studies, but with attenuated effect sizes (Satterthwaite et al., 2013). Further, a more recent study applying improved methods for motion artefact removal (Power et al., 2012; Patel et al., 2014) to resting-state fMRI data found no evidence of distance-dependent adolescent development of FC (Marek et al., 2015).

Other aspects of developing FC architecture were investigated, beyond the controversial distance-dependence of FC maturation. Hwang et al. (2013) reported that hubs were largely stable from late childhood to early adulthood, but that their connectivity was refined during adolescence. Specifically, an early increase in FC between integrative frontal hubs and cortical and subcortical non-hubs was followed by a later increase in connectivity between cerebellar hubs and cortical non-hubs (Hwang et al., 2013). Marek et al. (2015) found that the architecture of functional communities remained stable throughout adolescence, but that cross-network integration, particularly of a cingulo-opercular/salience network, increased with age. Conversely, Gu et al. (2015) reported an increase in the variance of community size as well as age-related changes in the balance of within- and between-module connectivity of specific systems, hypothesized to underlie the maturation of cognitive function. For a summary of the literature on adolescent development of whole-brain resting-state FC, see Table A.2 in Appendix A.

Furthermore, given hypothesised differences in the timing of subcortical and association-cortical adolescent maturation (Mills et al., 2014), multiple studies have focused on the development of subcortical FC. These studies reported heterogeneous findings of both decreases (Supekar et al., 2009; Gabard-Durnam et al., 2014; Fareri et al., 2015; Sato et al., 2015) and increases (Van Duijvenvoorde et al., 2015; Marek et al., 2015) in FC between specific subcortical regions and cortex; for a summary of the specific findings on adolescent maturation of subcortico-cortical FC, see Table A.3 in Appendix A. Importantly, most of these studies have focused on specific subcortical "seed" region(s) of interest, while the few that investigated subcortical connectivity more broadly have clustered subcortical regions into subnetworks, losing anatomical specificity.

Heterogeneity of findings across the literature, including seemingly opposite effects of age within similar circuits, might be due to variations in data processing steps. For example, some (but not all) of the aforementioned studies have applied global signal regression (GSR), which can lead to substantial changes in FC architecture (e.g.: Yan et al., 2013). Moreover, many of the above studies applying global "higher-order" graph-theoretical measures were conducted prior to the realisation that when these summary network properties are estimated
for networks constructed using Pearson’s correlation, they are strongly related to simple properties of the correlation distribution (van den Heuvel et al., 2017).

Here, in an attempt to address the heterogeneity of findings in the literature on FC development, and associated persistent uncertainty regarding normative maturational trajectories of functional architecture, we study FC maturation in a sample of 97 healthy adolescents aged 14-24 years. To adequately correct fMRI time series for the spurious effects of participant in-scanner motion, we used data acquired with this issue in mind. Resting-state scans were acquired using a multi-echo acquisition (Barth et al., 1999) and denoised using multi-echo ICA (ME-ICA; Kundu et al., 2012, 2013), a method designed to identify and discard noisy components of fMRI time series unrelated to the BOLD signal. After ensuring that FC is not related to motion across subjects, we focused on methods for the analysis of unthresholded (fully weighted) networks, to precisely characterise developmental effects on the full FC distribution. In addition to cortico-cortical connectivity, we comprehensively characterised the maturation of subcortico-cortical edges, including developmental profiles of connectivity between each individual subcortical region and all cortical regions.

We find that the distribution of functional correlations presents a non-uniform shift over adolescence, driven by adolescent strengthening of initially strong connections within primary sensorimotor and visual areas. Conversely, association cortical and subcortical edges undergo a subtler reorganisation of FC. Furthermore, individual subcortical regions show distinct maturational profiles, prominently involving association cortex. Finally, by partitioning regions developing at similar rates into maturational modules, we confirm the patterning of maturation according to known functional systems.

6.2 Methods

6.2.1 Participants

A demographically balanced cohort of 97 adolescents (47 females) aged 14-24 years was included in this study, with approximately 20 participants in each of 5 age-defined strata: 14-15 years inclusive, 16-17 years, 18-19 years, 20-21 years and 22-24 years. These participants are a subset of the 297 participants used to construct structural networks in Part II of this thesis. Participants provided informed written consent for each aspect of the study, and parental consent was obtained for those aged 14–15 years. The study was ethically approved by the National Research Ethics Service and was conducted in accordance with NHS research governance standards.


### 6.2.2 MRI acquisition and processing

Scanning took place at three sites, all operating identical 3T MRI systems (Magnetom TIM Trio, Siemens Healthcare, VB17 software version) with standard 32-channel radio-frequency (RF) receive head coil and RF body coil for transmission. Resting-state fMRI data were acquired using a multi-echo echoplanar imaging (ME-EPI) sequence with online reconstruction (Barth et al., 1999): repetition time (TR) = 2.42 s; GRAPPA with acceleration factor = 2; flip angle = 90°; matrix size = 64 × 64 × 34; FOV = 240 × 240 mm; in-plane resolution = 3.75 × 3.75 mm; slice thickness = 3.75 mm with 10% gap, sequential slice acquisition, 34 oblique slices; bandwidth = 2,368 Hz/pixel; echo time (TE) = 13, 30.55 and 48.1 ms. For pre-processing of these data, we used multi-echo independent component analysis (ME-ICA; Kundu et al., 2012, 2013) to identify the sources of variance in the fMRI time series that scaled linearly with TE and could therefore be confidently regarded as indicative of BOLD contrast. Other sources of fMRI variance, such as head movement, which were not BOLD-dependent and therefore did not scale with TE, were identified by ME-ICA and discarded. The retained independent components, representing BOLD contrast, were optimally recomposed to generate a broadband denoised fMRI time series at each voxel (Posse et al., 1999). This was bandpass filtered by the discrete wavelet transform (Daubechies 4 wavelet), resulting in a BOLD signal oscillating in the frequency range 0.025-0.111 Hz (wavelet scales 2 and 3).

During processing, re-alignment of scans was used to estimate 6 motion parameters for each participant (3 translation parameters and 3 rotation parameters). Subsequently, these were used to calculate an overall estimate of motion - the framewise displacement (FD), defined as the sum of the absolute derivatives of the six motion parameters, following conversion of rotational parameters to distances by computing the arc length displacement on the surface of a sphere with radius 50 mm (as in Power et al. (2012) and Patel et al. (2014)):

\[
FD_t = \sum_d |d_{(i-1)} - d_i| + 50 \cdot \frac{\pi}{180} \sum_r |r_{(i-1)} - r_i| \tag{6.1}
\]

where \(d\) denotes translation distances \((x,y,z)\), and \(r\) denotes rotation angles \((\alpha, \beta, \gamma)\). For each participant, mean FD was calculated by averaging the FD time series.

Each subject’s gray matter was parcellated into 324 regions of interest, including 308 cortical and 16 subcortical regions. The 308 cortical regions were obtained by sub-parcellating the Desikan-Kiliany anatomical atlas (Desikan et al., 2006) into parcels of approximately equal surface area (~5cm²; Romero-Garcia et al., 2012), and were the same regions as used in Part II. The 16 subcortical regions were provided by FreeSurfer software (Filipek et al.,...
1994), and included bilateral pairs of the following regions: thalamus, caudate, putamen, pallidum, hippocampus, amygdala, nucleus accumbens and the ventral diencephalon. Regional BOLD time series were estimated by averaging time series over all voxels in each of the 324 parcels. Some regions (particularly near the frontal and temporal poles) were excluded because of low regional mean signal, defined by a low Z-score of mean signal intensity in at least one subject (Z < -1.64); this resulted in the exclusion of 37 cortical regions, primarily in inferior temporal and pre-frontal cortex. Further, three participants were excluded because of poor co-registration between functional and anatomical data. Finally, FC matrices were constructed by cross-correlating the BOLD time series between pairs of the retained regions (using Pearson’s correlation), resulting in 97 individual matrices capturing pair-wise FC of 287 regions.

**Effects of motion on functional connectivity**

Prior to conducting analyses on FC development, we verified whether participants’ overall motion (quantified using time-averaged FD) changes as a function of age. Moreover, to evaluate potential residual effects of motion on FC, we calculated the correlation between FC at each edge and participants’ motion (across participants). Subsequently, to assess whether motion affected FC in a distance-dependent manner, we correlated the upper triangular part of this matrix (of correlations between FC and motion) to the upper triangular part of a matrix of Euclidean distances between the centroids of all regions, as in Satterthwaite et al. (2012).

**6.2.3 Maturation of functional connectivity**

Maturation of FC with age was modelled as linear, covarying for sex.

Linear models including an interaction term between age and sex presented poorer fits (as indicated by increased AIC) than models without the interaction term fitted to the same data. Moreover, none of the interaction models were significant; we therefore focused on effects of age and sex only.

Additionally, we fitted locally adaptive smoothing splines, better suited than quadratic models at modelling non-linear trajectories (Fjell et al., 2010). However, we found no substantial evidence of non-linearities (data not shown), and thus focused on linear trajectories.

Linear models were fitted at several scales: (i) globally, at the level of the correlation distribution, (ii) within and between cytoarchitectonic classes of the von Economo atlas (von Economo and Koskinas, 1925), (iii) regionally, using average nodal FC and (iv) edge-wise.
Global maturation of functional connectivity

To evaluate maturational shifts in the functional correlation distribution, we first visualised the individual distributions as a function of age. Subsequently, we summarised each individual distribution using its four moments: the mean, variance, skewness and kurtosis, fitting linear models as a function of age (covarying for sex) to each. Finally, to more precisely characterise potential local age-shifts in the correlation distribution, we fitted linear models to the value of correlation at each percentile of the distribution, and compared the effect sizes (slopes as a function of age) across percentiles.

Maturation of functional connectivity within cytoarchitectonic classes

We next studied the maturation of FC within cytoarchitectonic classes of the von Economo atlas (von Economo and Koskinas, 1925), which provides a useful frame of reference and means of dimensionality reduction of the data.

Initially, in addition to the seven classes used in Chapter 4, we considered an additional "class", consisting of the eight bilateral pairs of subcortical regions included in our anatomical atlas. We first calculated average FC for each subject within each class and between each pair of classes, before fitting linear models as a function of age (covarying for sex) to each set of within or between-class average weights. This resulted in estimates of FC at age 14 (the intercept of the linear model), and effect sizes for age, as well as sex, for each pair of cytoarchitectonic classes.

Subsequently, as we expected individual subcortical regions to show distinct profiles of subcortico-cortical maturation, we separately investigated the development of FC between each bilateral pair of subcortical regions and the seven classes of the von Economo atlas. Again, we fitted linear models as a function of age (covarying for sex) to subject-wise average FC of each set of edges, between each subcortical region and each cytoarchitectonic class.

Nodal maturation of functional connectivity

We next focused on individual regions using node strength, defined as the average FC of a node to all other nodes. In addition to this overall estimate of node strength, we recalculated node strength over subsets of cortical edges (between the 271 cortical regions and all 287 regions) and subcortical edges (between the 16 subcortical regions and all 287 regions).

For all three sets of nodal strength maps (all-all, cortical-all and subcortical-all), we characterised regional development of FC by fitting linear models of changes in node strength as a function of age, covarying for sex. This resulted in estimates of FC at age 14 (the
intercept of the linear model), and effect sizes for age, as well as sex, at each node (separately for all edges, cortical edges and subcortical edges).

Moreover, we constructed maps of subcortico-cortical maturation of FC for each of the eight bilateral pairs of subcortical regions listed above. Maps of the development of FC to each cortical node were obtained by fitting linear models to the average of the two edges between each bilateral pair of subcortical regions and each cortical region.

**Edge-wise maturation of functional connectivity**

Finally, the most fine-grained description of FC development was obtained by fitting linear models as a function of age to each edge (Pearson’s correlation) in the FC matrix. This led to matrices of (i) the edge-wise effect sizes of age ($\Delta$ age), (ii) edge-wise effects of sex, which was covaried for when fitting the linear models ($\Delta$ sex) and (iii) edge-wise FC at 14, corresponding to the intercept of the linear models. These matrices were then explored in several ways.

First, the edge-wise linear models were used to evaluate pairwise relationships between parameters extracted from these models, for all three pairs of parameters (FC at 14 VS $\Delta$ age, FC at 14 VS $\Delta$ sex, $\Delta$ age VS $\Delta$ sex). These relationships were quantified at the nodal level, using rows (or equally, columns) of the matrices of edge-wise parameters. The relationships were visualised by von Economo cytoarchitectonic class, as well as (for cortical nodes) on the cortical surface.

Furthermore, each of the three matrices was partitioned into modules using the Louvain multi-resolution community algorithm (Blondel et al., 2008). Similarly to Chapter 4, the algorithm was applied over the resolution parameter range $0.01 \leq \gamma < 4.00$, to identify resolution parameter values at which nodes are most consistently affiliated to the same module (Shinn et al., 2017). The final community partition was defined as a consensus across 1000 runs of the Louvain modularity algorithm (Lancichinetti and Fortunato, 2012) at the selected values (for each of the three matrices) of the resolution parameter $\gamma$. As in its application to traditional connectivity matrices, the algorithm leads to modules with strong edges located within modules and weak edges located between modules. Application of the algorithm to the matrix of FC at age 14 leads to modules of nodes which are strongly connected at baseline; however, the interpretation differs from traditional applications for the remaining two matrices. For the matrix of edge-wise effects of sex on FC, the resulting modules can be interpreted as sex modules, of nodes showing the most similar difference between males and females. For the matrix of effects of age on FC, modules correspond to maturational modules, of nodes developing at similar rates. Overlap between the three community partitions was quantified using the normalised mutual information (NMI). Similarly,
the NMI was used to quantify the overlap between each of the three partitions and the von Economo atlas of cytoarchitectonic classes (von Economo and Koskinas, 1925), as well as a template of intrinsic connectivity networks (Yeo et al., 2011). For the latter comparisons we excluded subcortical nodes from our three community partitions, as the two external community templates comprise only cortical nodes.

Finally, we inspected potential effects of the distance of functional connections on their maturation, by evaluating Spearman’s correlation between the upper triangular parts of the matrices of Euclidean distance and effects of age on FC.

6.3 Results

6.3.1 (In)dependence of functional connectivity on head motion

Given the prominent effects of participant in-scanner motion on past resting-state fMRI studies of adolescent development (see Introduction), we first focused on potential residual effects of this confounding variable.

We found no evidence of a change in participants’ overall in-scanner motion (quantified using time-averaged FD; equation 6.1) as a function of age (Spearman’s $\rho = -0.060$, $P = 0.56$; Fig. 6.1A).

Furthermore, we evaluated the correlation (across participants) between FC at each edge and participants’ average head motion. Similarly to previous studies (e.g.: Satterthwaite et al., 2012; Dipasquale et al., 2017), we found an average correlation of 0.25, indicating an overall increase in FC for subjects who move more (average along the y-axis in Fig. 6.1B). This relationship was also present when average FD and FC were correlated (Spearman’s $\rho = 0.40$, $P = 5.7 \cdot 10^{-5}$; Fig. 6.1C).

Using the aforementioned edge-wise correlation between motion and FC, we verified whether the impact of motion on edge-wise FC is modulated by the Euclidean distance spanned by these edges. We found a weak positive correlation that was highly significant, but likely so due to the high number of edges (Spearman’s $\rho = 0.090$, $P < 10^{-10}$; Fig. 6.1B). We note that in datasets where effects of motion were not adequately dealt with, this relationship is negative (such that correlations are decreased by motion for longer edges), and of a substantially higher magnitude (e.g.: $r = -0.50$ in Satterthwaite et al. (2012)). Thus, the distance-dependent impact of motion in these data appears to be minimal.

Still, to remove residual effects of motion on FC, and in particular the positive correlation between FC and motion across participants, we regressed average participant motion (mean FD) from FC at each edge. Corrected FC values are given by the combination of subject-
6.3 Results

Fig. 6.1 Effects of head motion on functional connectivity. A) Average participant motion (quantified as mean FD; equation 6.1) does not change with age. B) The correlation between FC at each edge and the corresponding participant’s motion (across participants) shows a weak relationship with the Euclidean distance spanned by edges. Moreover, the average edge-wise correlation between FC and motion (average of data along the y-axis) is non-zero. C) Mean participant motion is strongly related to mean FC across participants, such that the functional networks of subjects who move more are more strongly functionally connected. D) To remove the dependence of FC on motion, mean FD was regressed from each edge; the residuals constitute subject-specific FD-corrected FC, with intercepts retained to maintain the relative importance of edges across the group as well as the interpretability of FC values. Following edge-wise correction of FC for motion, the correlation between FC and motion vanished - both E) at the level of individual edges (by definition), and F) at the whole-brain level. Panels B and E depict scatterplot density of the underlying dense data, with two-dimensional hexagonal bins color-coded by the number of edges located within.
specific residuals and global intercepts, which were added to the residuals to maintain the relative importance of edges across the group as well as the interpretability of FC values (Fig. 6.1D). We note that this step has been previously applied for post-hoc correction of effects of motion (Gu et al., 2015).

Following this additional step, the correlation between FC and motion across participants vanished. At the edge level, the correlation becomes zero (by definition), due to regression at this level. Thus, distance-dependent effects of motion on FC also disappear (Fig. 6.1E). The relationship between motion and mean FC is also non-significant following this step (Spearman’s \( \rho = 0.017 \), \( P = 0.87 \); Fig. 6.1F).

We note that the application of this step has no qualitative effect on our results; it simply slightly increases effect sizes of changes in FC as a function of age (results not shown). Henceforth, all references to FC refer to the FD-regressed FC matrices.

### 6.3.2 Global maturation of functional connectivity

The FC distribution shifted over adolescence, as suggested visually (Fig. 6.2A) and as apparent in changes with age of its four moments (Fig. 6.2B). A slow rightward shift of the entire distribution is evidenced by a weak increase in its mean (Spearman’s \( \rho = 0.15 \), \( P = 0.031 \)). Further, an increase in variance of the correlation distribution suggests its widening with age (Spearman’s \( \rho = 0.36 \), \( P = 0.00070 \)), which given a decrease in kurtosis (Spearman’s \( \rho = -0.28 \), \( P = 0.0062 \)) appears to occur predominantly within the tails of the distribution. The distributions remained positively skewed (with a right-hand "heavy tail" indicative of strongly connected hubs) across the age range, presenting little evidence of change in skewness over adolescence (Spearman’s \( \rho = -0.059 \), \( P = 0.56 \)).

Potentially subtle changes in the correlation distribution can be more precisely characterised by fitting linear models to the value of correlation at each percentile of the distribution (Fig. 6.3A). This indicates an increasing rate of strengthening as a function of percentile, with the right tail of the correlation distribution strengthening fastest (Fig. 6.3B). Increases in correlation as a function of age are significant (Spearman’s \( P < 0.05 \)) at the 48th percentile, and as of the 50th percentile (median) upwards.

The above results are not driven by the application of a Fisher r-to-Z transform to the individual correlation distributions, as results remain qualitatively consistent without the application of this transform (results not shown).

Taken together, changes in the correlation distribution indicate a strengthening of FC over adolescence, together with an increase in the spread of FC values. The faster increase of the right tail of the distribution suggest an increase in the predominance of the strongest connections. However, the lack of anatomical specificity at this stage precludes disentangling
6.3 Results

Fig. 6.2 Global trajectories of the correlation distribution. A) Development of the functional correlation distribution of individual participants. Distributions are colour-coded by age. B) Trajectories of summary measures of the correlation distribution as a function of age: (i) mean, (ii) variance, (iii) skewness and (iv) kurtosis.

Fig. 6.3 Higher percentiles of the correlation distribution strengthen faster with age. A) Development of the functional correlation distribution of individual participants. Distributions are colour-coded by percentile. B) Higher percentiles of the correlation distribution strengthen faster, as indicated by increasing slopes of linear models fit to values of correlation at increasing percentiles. This is visualised using (i) plots of increases in correlation at three selected percentiles (50th, 90th, 99th), as well as (ii) a plot of all the linear models as a function of age (centered along the y-axis for visual clarity; left) and a plot of the coefficients (slopes) of the age-term within these linear models as a function of percentile (right).
between a process of adult strengthening of adolescent hubs, or adult emergence of new, stronger hubs.

6.3.3 Maturation of functional connectivity within cytoarchitectonic classes

We next investigated the maturation of FC within and between cytoarchitectonic classes of the von Economo atlas (Fig. 6.4A).

First, we investigated all cortico-cortical and cortico-subcortical edges by considering subcortical regions as an extra class, in addition to the seven cortical classes. Using linear models fitted to the subject-wise average FC of each within- or between-class edge set, we extracted maps of FC at 14 (the intercept of the linear model), the effect of sex and the effect of age (Fig. 6.4B). At age 14, FC was highest within classes 1, 4 and 5, corresponding mainly to primary visual and sensorimotor areas, as well as between these classes. Additionally, FC was high within classes 6 and 7, corresponding respectively to limbic and insular cortex, as well as within subcortical regions (Fig. 6.4Bi). Effects of sex were greatest within and between classes 1 and 5, corresponding to primary sensorimotor areas, as well as between class 7 (insula) and the rest of cortex, with increased FC in males compared to females; however, none of these effects survived FDR correction for multiple comparisons (Fig. 6.4Bii). Finally, effects of age were strongest within and between classes 1 and 5 (primary sensorimotor cortex), which showed the greatest increases in FC ($P_{FDR} < 0.05$). Other prominent effects of age occurred between these classes and class 4 (visual and pre-frontal areas), as well as between class 7 (insula) and the remaining cortical classes; however, these increases in FC did not survive FDR correction for multiple comparisons (Fig. 6.4Biii). Overall a pattern is apparent, whereby the same sets of (cortical) edges that are highly connected at age 14 show the greatest sex differences as well as the greatest increases with age - in other words, the strong edges are becoming stronger.

Subsequently, we studied the maturation of subcortico-cortical FC in greater detail, by fitting linear models as a function of age to FC between each of the seven cortical classes of the von Economo cytoarchitectonic atlas and each bilateral pair of subcortical regions (Fig. 6.4C). At age 14, the subcortical regions showing highest FC to cortex were the thalamus and caudate. The pallidum and putamen also showed high FC at 14, particularly to classes 1 and 5 (primary sensorimotor), 6 (limbic) and 7 (insula) (Fig. 6.4Ci). Effects of sex were greatest in the hippocampus, which showed increased connectivity to all cytoarchitectonic classes in males relative to females ($P_{FDR} < 0.05$ to classes 1 and 7). The amygdala and diencephalon also showed significant increases in male relative to female FC to certain
Fig. 6.4 Maturation of functional connectivity within and between cytoarchitectonic classes. A) A cortical map of cytoarchitectonic classes of the von Economo atlas. A classification into seven classes was used, including the original five classes proposed by von Economo (von Economo and Koskinas, 1925), as well as additional classes for limbic cortex (class 6) and insula (class 7; Vértes et al., 2016). B) Parameters of within and between-class FC, including all subcortical regions as an additional class, extracted from linear models: (i) FC at 14, (ii) effects of sex (as male - female), and (iii) effects of age. C) Parameters extracted from linear models fitted to FC between bilateral pairs of individual subcortical regions and each of the seven cortical classes of the von Economo atlas: (i) FC at 14, (ii) effects of sex (as male - female), and (iii) effects of age. In panels B and C, the size of cells along the von Economo dimensions of the matrices is proportional to the number of nodes in each class. Subcortical regions are listed in decreasing order of average rate of change with age. In panels (ii) and (iii), markers indicate significance of the corresponding coefficients of the linear models. (dc = ventral diencephalon)
classes, although these changes did not survive FDR correction for multiple comparisons (Fig. 6.4Cii). Finally, increases in FC with age were greatest for the hippocampus, amygdala and ventral diencephalon, to most classes except class 4; however, these increases did not survive FDR correction for multiple comparisons (Fig. 6.4Ciii). Changes in FC between the remaining subcortical regions (pallidum, putamen, accumbens, caudate, thalamus) and cortex were of smaller magnitude; however, these regions presented striking distinctions in maturation of connectivity to primary cortical classes (1, 4 and 5), which showed little change or decreased, and to association cortical classes (2, 3, 6 and 7), which tended to increase.

We also note that the relationship between FC at 14 and age- and sex-related changes differs from cortico-cortical connectivity: in subcortex, the greatest increases in FC in males and as a function of age are shown by subcortical regions that are weakly connected at age 14 (and vice-versa).

### 6.3.4 Nodal maturation of functional connectivity

Nodal maturation of FC was investigated by fitting linear models (covarying for sex) to average regional correlation or node strength. In addition to an overall estimate of node strength, obtained by averaging over all of a region’s edges, separate nodal contributions to cortical and subcortical node strength were obtained. Maps of overall node strength were

**Fig. 6.5 Regional maturation of functional networks.** Left to right: node strength at age 14, effect of sex on node strength (as m-f) and effect of age on node strength; the top row depicts cortico-cortical node strength, the bottom row depicts subcortico-cortical node strength. Full node strength (including both cortico-cortical and cortico-subcortical edges) is not depicted; its cortical maps appear near-identical to the cortico-cortical maps.
6.3 Results

near-identical to maps of cortical strength (Spearman’s $\rho = 1$ between corresponding pairs of maps), likely due to the prevalence of cortical nodes (271/287) relative to subcortical nodes (16/287). To avoid redundancy, maps of overall node strength were therefore not visualised.

Nodes in primary occipital and parietal cortices as well as superior temporal cortex presented strongest FC at age 14 (Fig. 6.5, left). This was evident within both cortico-cortical and subcortico-cortical networks, which at baseline showed high similarity (Spearman’s $\rho = 0.43$, $P < 10^{-10}$).

Males generally presented stronger FC than females, predominantly in lateral frontal and parietal regions (Fig. 6.5, middle). Many of these differences were significant, both within maps of cortico-cortical node strength ($P_{FDR} < 0.05$ at 200/287 regions) and within maps of subcortico-cortical node strength ($P_{FDR} < 0.05$ at 32/287 regions). Maps of cortico-cortical and subcortico-cortical effects of sex presented moderate overlap (Spearman’s $\rho = 0.26$, $P = 8.7 \cdot 10^{-5}$).

Effects of age presented the main signal of interest (Fig. 6.5, right). Nodes in primary occipital and parietal cortices and superior temporal cortex presented greatest increases in cortico-cortical FC over adolescence. Subcortico-cortical edges presented a different maturational profile, prominently involving association cortical connections. Many of these changes were significant prior to FDR correction for multiple comparisons (cortico-cortical: $P < 0.05$ at 106/287 regions, subcortico-cortical: $P < 0.05$ at 31/287 regions), although no region reached statistical significance after FDR correction.

A maturational divergence between sets of cortical and subcortical edges is evidenced by differential relationships of cortico-cortical and cortico-subcortical maturational trajectories to the corresponding functional architectures at age 14. Within cortex, regions which are strongly connected at age 14 show greatest increases in FC over adolescence (Spearman’s $\rho = 0.12$, $P = 0.041$), while the greatest increases in subcortico-cortical FC over adolescence are exhibited by nodes that show weak subcortico-cortical FC at age 14 (Spearman’s $\rho = -0.25$, $P = 1.3 \cdot 10^{-5}$). Accordingly, maps of cortico-cortical and cortico-subcortical FC development are weakly (albeit nonsignificantly) anti-correlated (Spearman’s $\rho = -0.10$, $P = 0.084$).

Maturation of individual subcortical regions

Following evidence of differences in cortical and subcortical development, we wished to map maturational trajectories of individual subcortical regions in greater detail. We therefore fitted linear models as a function of age (covarying for sex) to the average of two edges, between each bilateral pair of subcortical regions and each cortical region.
Notably, while many age-related changes and sex differences were significant prior to FDR correction for multiple comparisons, only the hippocampus showed significant strengthening of connectivity to cortex after FDR correction, and only the hippocampus, diencephalon and putamen showed sex differences after FDR correction (Table 6.1).

Regional trajectories of subcortico-cortical maturation confirm the substantial heterogeneity between maturation of individual subcortical regions that was previously seen at the level of cytoarchitectonic classes, but provide additional anatomical specificity. Again, the subcortical regions generally showing weaker FC to cortex at age 14 (e.g.: hippocampus, amygdala, diencephalon) show greater FC in males relative to females, and greatest increases as a function of age.

6.3.5 Edge-wise maturation of functional connectivity

To further characterise adolescent maturation of FC, linear models were fitted to individual edges (covarying for sex). This led to three matrices of edge-wise effect size: matrices of edge-wise FC at 14, edge-wise effects of sex and edge-wise effects of age.

Association-cortical and subcortical "re-ranking" of functional connectivity

Analyses conducted above point to regionally specific relationships between baseline architecture of FC, and its changes as a function of age and sex. In particular, within cortex, regions that are strongly connected at age 14 seem to further strengthen their connectivity into adulthood and show the greatest differences between males and females, while subcortico-cortical edges that are strong at age 14 show the smallest magnitude of change with age and the smallest sex differences. To obtain further anatomical specificity regarding these relationships, we evaluated Spearman’s correlations between edge-wise baseline architecture and its sex- and age-related changes at each node, using pairs of edge-wise maps (of FC at 14, Δ sex and Δ age; Fig. 6.7B). We visualised these relationships within cytoarchitectonic classes of the von Economo atlas, as well as (for cortical nodes) on the cortical surface.

First, relationships between FC at 14 and its difference between sexes show predominantly negative relationships (Fig. 6.7 C-D left), indicating that edges that are weakly connected at age 14 show the greatest increase in males relative to females, and vice-versa. These relationships are most negative for edges originating in association-cortical class 2, as well as in limbic cortex (class 6). Primary sensorimotor areas (classes 1 and 5) show positive relationships, indicating that those edges that show strong connectivity at age 14 are more strongly connected in females than males.
6.3 Results

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>increase</td>
<td>P &lt; 0.05</td>
<td>283</td>
<td>282</td>
<td>279</td>
<td>231</td>
<td>243</td>
<td>243</td>
<td>231</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>PrDR &lt; 0.05</td>
<td>122</td>
<td>95</td>
<td>8.3</td>
<td>18</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>decrease</td>
<td>P &lt; 0.05</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PrDR &lt; 0.05</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Δ age</td>
<td></td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>56</td>
<td>44</td>
<td>119</td>
<td>119</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P &lt; 0.05</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PrDR &lt; 0.05</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>increase</td>
<td>P &lt; 0.05</td>
<td>178</td>
<td>103</td>
<td>81</td>
<td>19</td>
<td>73</td>
<td>4</td>
<td>4</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>PrDR &lt; 0.05</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>decrease</td>
<td>P &lt; 0.05</td>
<td>9</td>
<td>7</td>
<td>20</td>
<td>41</td>
<td>4</td>
<td>23</td>
<td>23</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>PrDR &lt; 0.05</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6.1 Frequency and significance of age and sex-related changes in subcortico-cortical connectivity. Frequencies of positive and negative effects of age and sex on subcortico-cortical FC are listed, corresponding respectively to increases or decreases as a function of age (Δ age) and increases in males (♂) or females (♀; Δ sex). These frequencies are listed overall (regardless of significance), as well as after taking statistical significance into account - both without and with correction of P-values for multiple comparisons. Subcortical regions are listed in decreasing order of average rate of change with age.
Fig. 6.6 Maturation of subcortico-cortical functional connectivity for individual subcortical regions. Parameters of linear models extracted from subcortico-cortical FC, averaged across the two edges between each bilateral pair of subcortical regions and each cortical region. Effects of age (top row), effects of sex (middle row), and effects of age x (bottom row). Maps are ordered from left to right in decreasing order of average rate of change with age. Due to space constraints as well as strong bilateral symmetry exhibited by the full structural dataset, only the left hemisphere is visualized.
Fig. 6.7 Relationships between edge-wise maps of FC at 14, Δ sex and Δ age within cytoarchitectonic classes. A) A cortical map of cytoarchitectonic classes of the von Economo atlas. A classification into seven classes was used, including the original five classes proposed by von Economo (von Economo and Koskinas, 1925), as well as additional classes for limbic cortex (class 6) and insula (class 7) (Vértes et al., 2016). Subcortical regions were considered as an additional "eighth" class. B) Relationships between pairs of edge-wise maps were evaluated using Spearman correlations between rows of the corresponding matrices. These relationships are visualised C) by cytoarchitectonic class, as well as D) on the cortical surface. In C, the violin plots are coloured by average Spearman’s ρ within the corresponding cytoarchitectonic class.
Next, the map of the relationship between FC at age 14 and its adolescent change (Fig. 6.7 C-D middle) confirms previous indications of a "re-ranking" of association-cortical and subcortico-cortical connectivity. A similar effect is seen within limbic cortex (class 6). Conversely, edges between primary and secondary sensory classes (1, 4 and 5) as well as the insula (class 7) are showing positive correlations, indicating a strengthening of already strong edges.

Finally, the map of relationships between changes with age and sex differences shows only positive correlations (Fig. 6.7 C-D right), indicating that similar edges show increases as a function of age as those that show the greatest differences between males and females. These relations are strongest for primary sensorimotor regions (classes 1 and 5), followed by subcortex.

**Communities of similar edge-wise effects**

Further, we decomposed the matrices of edge-wise FC at age 14, effects of sex and effects of age into communities of nodes showing similar effects. To this end, we applied a multi-resolution community detection algorithm (Blondel et al., 2008) to these three matrices, using the concept of minimising versatility (Shinn et al., 2017) to identify values of the community resolution parameter $\gamma$ at which nodes were most consistently assigned to the same module. Note that no single "optimal" local minimum of node versatility exists for each matrix, and correspondingly no optimal number of modules.

We chose different local minima for each of the three matrices, which yielded different numbers of modules. The matrix of FC at 14 was partitioned into 3 modules, the matrix of edge-wise effects of sex into 4 modules and the matrix of edge-wise effects of age into 5 modules (Fig. 6.8). All three sets of communities included an occipital "visual" module (green modules in Fig. 6.8), a superior "somato-sensory" module (yellow/orange modules in Fig. 6.8) and a fronto-parietal "association" module (red modules in Fig. 6.8). Additionally, the sex and maturational modules included respectively one and two distributed communities, one of which prominently involves the cingulate gyrus (for maturational modules; blue in Fig. 6.8, right). However, the communities also showed differences: for example, subcortical nodes were distributed across modules at 14, but were more homogeneously assigned to the prefrontal module within community decompositions of sex and age-related change (Fig. 6.8, coloured dots in middle row).

The three sets of communities showed moderate overlap, with pair-wise NMI ranging from 0.21 (FC at 14 VS $\Delta$ sex), through 0.23 ($\Delta$ sex VS $\Delta$ age), to 0.31 ($\Delta$ age VS FC at 14). We also evaluated the overlap of the three sets of communities to the von Economo atlas of cytoarchitectonic classes (von Economo and Koskinas, 1925) and an external template
Fig. 6.8 **Communities of nodes that are strongly connected at baseline, show similar sex differences and mature at similar rates.** Top row: In the cortical plots, nodes are colored according to community affiliation; affiliation for subcortical nodes is indicated by the colored dots below. Alluvial diagrams between cortical surface plots indicate community overlap. Bottom row: Within matrices of edge-wise effects, communities are ordered (from top left to bottom right) in decreasing order of within-module average effect size. Further, within communities, nodes are ordered (from top left to bottom right) in decreasing order of average effect size.
of intrinsic connectivity networks (Yeo et al., 2011). Our three sets of edge-wise communities showed moderate overlap with the von Economo atlas of cytoarchitectonic classes (NMI = 0.27, 0.28, 0.19 respectively for FC at 14, Δ sex, Δ age), and particularly strongly with the external template of intrinsic connectivity networks (Yeo et al., 2011; NMI = 0.39, 0.53, 0.36 respectively for FC at 14, Δ sex, Δ age).

(In)dependence of maturation on distance

Finally, we inspected potential effects of the distance spanned by functional connections on their maturation. Upper triangular parts of matrices of Euclidean distance and effects of age on FC showed a weak negative relationship that was highly significant, although likely due to the high number of edges being compared (Spearman’s $\rho = -0.065$, $P < 10^{-10}$). The very small effect size indicates a likely lack of effects of distance on edge-wise maturation; still, the sign of the relationship indicates a possible preferential strengthening of short edges with age.

6.4 Discussion

In this study, we set out to describe maturational trajectories of human functional network architecture. We used resting-state fMRI scans of 97 healthy adolescents and young adults (aged 14-24 years), deliberately acquired and processed with the intention of removing spurious effects of participant in-scanner motion. We found that the distributions of functional correlations increase in an inhomogeneous manner, as already strong primary-cortical connections to the rest of cortex strengthen further into young adulthood. Conversely, association-cortical and subcortical edges undergo a subtler reorganisation of FC. Individual subcortical regions show distinct developmental profiles of connectivity to cortex, including wide-spread increases in FC, selective increases to association cortical areas or decreases in FC to sensorimotor regions. Finally, a partition of regions developing at similar rates yielded maturational modules strongly reminiscent of canonical resting-state networks, suggestive of developmental patterning according to known functional systems.

6.4.1 Impact of motion on estimates of functional network development

Many early studies of adolescent functional network maturation focused on distance-dependent effects, reporting preferential strengthening of long edges combined with weakening of short edges (Fair et al., 2007, 2009; Supekar et al., 2009; Dosenbach et al., 2010), interpreted
6.4 Discussion

As a shift from a 'local' architecture of anatomically proximal communities of nodes to a 'distributed' network architecture in young adulthood. However, these findings were likely driven by the fact that motion decreases correlation within long edges, and increases correlation within short edges (Satterthwaite et al., 2012). This, together with the fact that younger participants tend to move more in the scanner, could give rise to the artefactual finding of a preferential strengthening of long edges.

Following the realisation that these popular theories of distance-dependent functional maturation might be attributable to motion, several efforts were made to verify these findings. An initial re-analysis of distance-dependent maturation in subsamples matched for motion as well as samples where effects of motion were regressed out from FC, suggested that functional maturation may be distance-dependent, but that the magnitude of these effects is substantially reduced by taking motion into account (Satterthwaite et al., 2013). Subsequently, Marek et al. (2015) applied a combination of improved methods for motion artefact removal (Power et al., 2012; Patel et al., 2014) to resting-state fMRI data, and found no evidence of an interaction between the length of functional connections and their rate of change. Specifically, the authors failed to identify a difference in the Euclidean distances spanned by equally-sized sets of the most strengthening and weakening edges, nor did they find a significant correlation between edge-wise changes in correlation strength and distance (Marek et al., 2015).

In the present study, we find a significant but very weak relationship between the Euclidean distance spanned by edges and their adolescent rate of change. This relationship is negative, such that short edges show a tendency to increase in strength more over adolescence than long edges. However, the tiny effect size of this relationship warrants caution in interpretation of these results. In any case, the direction of the weak distance-dependent effect of functional maturation aligns with a recent study (Marek et al., 2015) supporting the view that early reports of increased maturation of long edges were caused by motion-related artefacts.

6.4.2 Development of subcortico-cortical functional connectivity

Our study reported marked differences in rates of change between well-defined systems. Primary cortical regions increased in their functional connectivity to each other and to the rest of cortex, while showing little net change in their connectivity to subcortex. Conversely, while association cortical regions show little net change in connectivity to the rest of cortex, their connectivity to subcortical regions increases over adolescence. Clear patterning of subcortico-cortical FC development is further apparent within maturational trajectories of the connections of individual subcortical regions. While hippocampus and diencephalon show indiscriminate increases in FC, and the nucleus accumbens shows little net change, the
remaining regions show anatomically-defined profiles of maturation, including distinct rates of change in FC to primary and association regions.

Although few of our maturational trajectories of subcortico-cortical FC are significant, it is interesting to compare the patterns of local increases and decreases in FC of individual subcortical regions to cortex to the existing literature - with which our findings present both agreement and discord. For example, Greene et al. (2014) reported decreases in connectivity of the pallidum and putamen to somatosensory cortex; this agrees with our results. Further, Gabard-Durnam et al. (2014) focused on the amygdala, reporting a combination of decreases (to insula, superior temporal sulcus, parahippocampal gyrus, posterior cingulate) and increases (to medial pre-frontal cortex) in FC. The latter increase aligns with our results of wide-spread increases in FC between amygdala and cortex; however, although our increases in FC include association regions, they are most prominent to primary areas. Subsequently, Fareri et al. (2015) reported adolescent decreases in connectivity of the ventral striatum (which includes the nucleus accumbens, ventral caudate and putamen) to medial prefrontal cortex - which in our data presents no clear changes in connectivity to the ventral striatum. Further reports of increasing FC of the caudate and thalamus to the dorso-lateral prefrontal cortex, along with increases in FC between the nucleus accumbens and the dorsal anterior cingulate (Van Duijvenvoorde et al., 2015), also do not align with our results. Additional studies inspecting adolescent maturation of subcortical FC are harder to compare to our results. Some have applied graph-theoretical measures to subnetworks of subcortical nodes (Supekar et al., 2009; Marek et al., 2015; Gu et al., 2015) or evaluated connectivity of an individual region relative to the whole network (e.g.: using eigenvector centrality; Sato et al., 2015), while others have relied on task-based fMRI data (e.g.: Cignetti et al., 2016). For a complete survey of adolescent developmental changes in subcortico-cortical resting-state FC, see Table A.3 in Appendix A.

Heterogeneity within the above set of findings, or disagreement with our results, may be explained by different choices of processing methods (including for example application of GSR by some studies but not others) or analytical approaches (whereas many studies apply seed-based voxel-wise analyses, some apply graph-theoretical methods). Still, our findings are in agreement with several of the above studies. Moreover, our analyses provide, for the first time, a comprehensive characterisation of maturational trajectories between each subcortical region and all remaining cortical regions, that should be provide a useful comparator to future studies of subcortical structure and function.
6.4 Discussion

6.4.3 Adolescent maturation is patterned by known intrinsic networks

By applying community detection methods to a matrix of developmental effects on the strength of individual functional connections, we identified a set of maturational modules that show high overlap with known resting-state networks (Yeo et al., 2011), as well as cytoarchitectonic classes of the von Economo atlas (von Economo and Koskinas, 1925). In line with the aforementioned strengthening of connectivity between many subcortical regions and association cortex, most subcortical regions (except for the left amygdala) were assigned to a module situated within frontal and parietal association cortex.

Resting-state networks have been hypothesized to result from the strengthening of connections due to task-based co-activations (Sporns, 2013), based on several studies demonstrating overlap between resting-state and task-evoked functional networks (Smith et al., 2009; Laird et al., 2011; Crossley et al., 2013) – a mechanism which has been described as a network-level extension of Hebbian plasticity (Sporns, 2013). However, in view of the present results, this putative network-level Hebbian mechanism can also be interpreted from a developmental perspective, whereby macroscopic brain regions might be strongly connected on account of sharing similar developmental trajectories.

It has recently been demonstrated that several canonical functional networks display correlated gene expression profiles across the lifespan (Richiardi et al., 2015), which could be responsible for the synchronous development of functional connectivity subnetworks in adolescence, as revealed here. It could thus be hypothesized that abnormalities in regional synchrony of functional maturation, themselves potentially driven by genetic aberrations (e.g.: Heck et al., 2014), will result in a “dysconnected” architecture of the functional connectome in adulthood. Specifically, such developmental aberrations might be focused within one specific maturational module. Indeed, it has recently been shown, within structural data, that childhood-onset schizophrenia displays developmental aberrations which are focused within a single maturational cluster of the healthy adolescent brain (Alexander-Bloch et al., 2014).

6.4.4 Limitations and future work

One limitation of this study is the relatively small sample analysed. Together with the high variability in FC across participants, this means that many of the developmental trajectories reported in this study are either not statistically significant, or do not survive FDR correction for multiple comparisons. The anatomical patterning of our findings, which in most cases respects known boundaries between primary and association regions, suggests that these results are meaningful. Still, the present results should be considered as exploratory, subject
to validation in an independent dataset. The remaining N = 200 resting-state fMRI scans of the NSPN study of normative adolescent development will provide an ideal dataset for this effort.

Furthermore, we found residual signatures of motion in our data, including relationships between average participant motion and FC at several spatial scales. These relationships, indicating that brain networks of subjects that move more tend to be more strongly functionally connected, are concerning and suggestive of the need to further improve algorithms for BOLD signal denoising. In the present work, we have removed these residual effects using regression, as has been applied previously (Gu et al., 2015). We note that application of this additional step does not qualitatively affect our results.

Finally, our study did not inspect behavioral or cognitive implications of the reported findings. Investigating such relationships is crucial if studies on normative development such as this one are to impact policy or clinical practice.

6.4.5 Conclusion

Functional connectivity undergoes substantial adolescent changes, with differential matura-
tion of FC in primary and association cortex, as well as the FC of these regions to subcortex. Our results indicate substantial reorganisation of association-cortical FC, consistent with the concept of tuning of association-cortical and sub-cortical connectivity in the adolescent functional connectome.
CHAPTER 7

SUMMARY AND CONCLUDING REMARKS

7.1 Summary

The studies presented in this thesis all show changes in correlation-derived estimates of structural or functional inter-regional brain connectivity. I have taken a range of methodological approaches to characterise these changes.

In Part I, schizophrenia was studied as a model disease which is known to present reductions in cross-correlations between regional BOLD signals. I have applied a novel probabilistic thresholding procedure, taking into account the regionally-dependent effects of motion on estimates of confidence in BOLD time series (quantified using effective $df$). In Chapter 2, I used this method to show increased disconnectivity of the functional connectome in schizophrenia, and compared probabilistic thresholding to traditional weight-based thresholding. Different edges were retained in order of increasing $df$-adjusted P value and in order of increasing weight (correlation). Moreover, the probabilistically thresholded edges were more consistent across participants. In Chapter 3, I studied the consequences of probabilistic thresholding for fixed-density network analysis. Although only a small proportion of edges differs between networks thresholded based on probabilities or weights, these edges lead to significant differences in summary topological organisation. Specifically, edges which are more likely following adjustment of P values for effective $df$ tend to be less random than stronger (but less likely) edges. Moreover, thresholding networks to fixed edge density while controlling for type I error, by only comparing subsets of participants whose edges are all statistically significant at each density, eliminates group differences in topological randomisation between healthy controls and patients with schizophrenia. This aligns with other recent work (van den Heuvel et al., 2017), suggesting that previously reported increases in network randomness in schizophrenia were methodologically grounded.
in retention of a fixed proportion of edges based on decreasing edge weight (correlation),
ignoring between-group differences in the correlation distribution and edge probability.

In Parts II and III, the NSPN study on normative adolescent development provided a "discovery" dataset to develop new analysis methods on. Healthy brain maturation during adolescence is still relatively poorly understood, but adolescent maturational aberrations are thought to underlie the frequent emergence of psychiatric diseases in this period (Paus et al., 2008).

In Chapter 4 I described healthy development of structural correlation (covariance) networks, constructed using cross-correlations between regional estimates of cortical thickness across participants. To improve upon previous studies which described changes in network correlations using discrete age bins (Zielinski et al., 2010; Khundrakpam et al., 2013), I applied a novel sliding-window method, which provides a more continuous representation of structural network development. Further, I thresholded structural networks using bootstrap resampling of participants within each window, retaining edges that were most consistent across samples and obtaining a more precise description of network maturational trajectories. Network connectivity developed in a nonlinear manner, with an initial steep decrease from 14 to approximately 19 years, followed by a slower increase to 24 years. This was driven by maturation of prefrontal and association-cortical areas, where changes were greatest and converged latest. In Chapter 5, I explored the sensitivity of these results, showing that they were qualitatively independent on parameters of the sliding window method or the models used to fit maturational trajectories. Moreover, I ascertained that these results were not driven by artefacts, including regions with low reliability of structural correlations or irregularities in the age distribution of participants.

Finally, in Chapter 6, I described adolescent maturation of functional brain networks, using a subset of the data studied in Part II. Focusing on simple network measures derived from dense, fully weighted networks enabled me to comprehensively characterise developmental shifts in network correlations. Globally, networks presented increases in the mean and spread of correlations, with the strongest edges strengthening fastest. Regionally, these increases appeared to be driven by strengthening of the connectivity of primary-cortical regions; however, closer inspection of trajectories within association-cortical areas revealed a subtler re-organisation of functional architecture, including increases in connectivity to subcortex. Furthermore, individual subcortical regions showed distinct profiles of subcortico-cortical maturation. Finally, decomposition of edge-wise rates of change into maturational modules revealed that adolescent development is patterned according to known resting-state networks.
7.2 Convergent themes

7.2.1 Correlation-derived brain networks

Correlation-derived brain networks, whether structural or functional, bear similarities. For example, the use of the correlation coefficient in their construction leads to statistical regularities such as increased transitivity (Zalesky et al., 2012; Hosseini and Kesler, 2013). These regularities subsequently give rise to relationships between mean correlation and higher-order measures of network organisation, when these networks are thresholded to fixed edge density by decreasing correlation (van den Heuvel et al., 2017). An outstanding question concerns the dependence of the strength of these relationships (between properties of the correlation distribution and higher-order network measures) on properties of the underlying data used to estimate structural or functional correlations. Examples of factors that might affect the strength of these relationships include the number of datapoints that are being correlated (the number of time-points in functional correlation networks, or number of participants in structural correlation networks), whether the mean of regional values was regressed prior to cross-correlation (global signal regression in functional networks, or correction for mean morphology in structural networks) or which measure of network organisation is being evaluated (some measures might be more strongly dependent on properties of the correlation distribution than others). These questions constitute important avenues for future work.

Despite similarities between structural and functional correlation brain networks, correlation-derived brain networks are not all created equal. A key difference is the estimation of correlations within subjects when constructing functional brain networks, compared to estimation of correlations across subjects when constructing structural (covariance) brain networks. While within-subject estimation of functional correlations yields individual brain networks which may facilitate single-subject inference, the time-series are readily confounded by within-subject sources of noise such as in-scanner motion or respiration, and present additional statistical structure such as temporal autocorrelations (Arbabshirani et al., 2014). Conversely, the group nature of structural (covariance) networks may impose limits on statistical inference, but the independence of data-points used to construct such networks is a notable advantage. Beyond enabling the application of simple permutation methods (as described in Part II), this independence is probably beneficial to the signal-to-noise ratio of inter-regional associations, as noise is likely to differ across subjects. The limiting group nature of structural covariance networks may be addressed by recently proposed methods for the construction of single-subject structural networks based on cross-correlation of morphometric features from multiple modalities (Seidlitz et al., 2018). To maximise their robustness, the number
of distinct modalities used to construct such morphometric similarity networks should be maximised, resulting in a greater number of (maximally independent) features, less affected by sources of structured noise which may affect multiple features extracted from a single acquisition or modality.

7.2.2 Adolescent development of structural and functional brain networks

Beyond general similarities and differences between structural and functional brain networks constructed using correlations, present regardless of the data used to construct these networks, relationships between these distinct modalities exist in the context of a single dataset, driven by the close link between structure and function of individual brains. As both structural and functional networks of developing adolescent brains were described within this thesis, it is worth considering the overlap between structure and function in these data.

First, it is notable that the locations of regions exhibiting fastest rates of change as a function of age differ in both datasets. Structural networks studied in Part II exhibited most prominent changes within association cortical areas, while showing little change in primary cortex. Conversely, functional networks analysed in Part III showed strongest rates of maturation in somatosensory areas. Moreover, the directions of these changes differed: while structural correlations in association cortex decreased, functional correlations in primary cortex increased, with functional reorganisation in association cortex exhibiting a combination of increases and decreases. An attempt at reconciliation of these findings would imply that functional connectivity increases (over adolescence) even in regions showing little apparent structural network change, while areas showing decreases in structural network connectivity exhibit concurrent re-organisation of functional network architecture. The generality of such changes could be investigated in other multimodal developmental datasets, including over different age-ranges. The presence of distinct changes in structural and functional architecture is likely not specific to developmental data; for example, different alterations in structural and functional brain networks have been reported in schizophrenia (Fornito and Bullmore, 2015).

To better understand similarities and differences between adolescent structural and functional brain networks, these modalities will need to be directly related. The simplest approach will be to extend functional network analyses presented in Part III to all (~300) participants of the NSPN study of adolescent development used to construct structural networks in Part II, before directly quantifying the overlap between resulting maps of regional network change. This constitutes an avenue for future work, together with the extension
of structural network analyses to morphometric features such as grey matter volume or surface area that are defined for subcortical regions, enabling examination of confluence (or divergence) between structural and functional network maturation in these key regions.

Further opportunities to directly relate structural and functional networks rely on extraction of other types of structural networks in individual subjects, including morphometric similarity networks (Seidlitz et al., 2018) or structural networks constructed using diffusion imaging (Jbabdi et al., 2015). Structural networks based on diffusion-weighted imaging can be further distinguished into individual networks constructed using computational tractography (Maier-Hein et al., 2017), or networks of voxel-wise diffusion-derived statistics (such as fractional anisotropy or mean diffusivity) averaged within specific tracts defined using a white matter atlas (Mori et al., 2009; Yeh et al., 2018). Following understanding of normative adolescent maturational trajectories of these types of structural networks, it will be important to study their relationship to developmental trajectories of functional networks described in Part III of this thesis, but also to maturation of structural covariance networks described in Part II, in order to obtain a holistic understanding of concurrent developmental changes in adolescent structural and functional network architecture.

Importantly, to disentangle causal relationships between changes in structure and function, these modalities will need to concurrently be analysed in longitudinal data (Foulkes and Blakemore, 2018), including the NSPN developmental data analysed herein. It is worth noting that the coupling between structure and function is itself likely dynamic and the nature of the relationship may show qualitative change over adolescence, which may complicate the evaluation of (causal) relationships between these modalities.

### 7.2.3 Development and disease

Intimate links exist between developmental changes in brain organisation, and the emergence of psychiatric diseases; indeed, numerous diseases emerge as a result of abnormal maturation (Silbereis et al., 2016). This is a key reason for the importance of studies on normative adolescent brain development, as characterisation of healthy maturational trajectories will help our ability to identify developmental aberrations.

Although schizophrenia was here studied as a model disease exhibiting decreases in functional correlations, it is a specific example of a psychiatric disorder which often emerges in adolescence (Paus et al., 2008). However, vulnerability to schizophrenia may be conferred much earlier still, including already during gestation (Selemon and Zecevic, 2015). Generally, the earlier a disease can be identified and treated, the better; thus, it will be important to extend our understanding of both healthy and abnormal maturation to wider age-ranges, including coverage up to newborn and even gestational stages.
Summary and concluding remarks

Crucially, understanding of relationships between adolescent development and disease will require the analysis of a range of cognitive and clinical questionnaires, and study of their links to network neuroimaging phenotypes. Ideally, such relationships should be studied in longitudinal data. Beyond NSPN, other large-scale efforts are aiming to shed light on such relationships, including the Adolescent Brain Cognitive Development study (ABCD; Volkow et al., 2017). Sharing of data and expertise amongst (and between) large consortia will be crucial to address such complex questions.

7.3 Generalisability of contributions

All methods discussed within this thesis pertain to construction and analysis of correlation-derived brain networks. While these methods are potentially generalisable to a wide range of scenarios, applications presented in this thesis all aim to address a common theme: shifts in the weight distribution of brain networks constructed using correlations, herein due to development or disease, carry implications for subsequent characterisation of "higher-order" network properties.

7.3.1 Probabilistic thresholding

Probabilistic thresholding approaches used in Parts I and II both lead to a "decoupling" of edge probabilities from weights (correlations). When thresholding the network by decreasing probability, this means that the strongest edges are not necessarily the most likely to be retained. Application of the probabilistic thresholding methods described in Part I is contingent on estimation of spatially heterogeneous loss of df due to motion, which can currently only be carried out with wavelet despiking. Thus, such subject-specific probabilistic thresholding is applicable to any functional connectivity data processed with methods presented in Patel and Bullmore (2016). Conversely, probabilistic thresholding approaches presented in Part II are applicable to any structural corelation network, following estimation of edge-wise uncertainty using bootstrap.

Beyond these instances of probabilistic thresholding, the idea of retaining connections based on some criterion of connection likelihood (rather than assuming that the strongest edges are the most likely) is widely applicable. Indeed, similar ideas have previously been applied to structural connectomes derived from diffusion imaging and tractography, including retention of edges which are consistently detected (de Reus and van den Heuvel, 2013) or consistently strong (Roberts et al., 2017) across participants. Such methods help constrain the choice of threshold, which is arguably more intuitive and less arbitrary in the probability
domain, and yield smoother distributions of edge weights with a gradual increase in the frequency of weaker edges, rather than a sharp "cut-off" as generated by weight-based thresholds.

Moreover, controlling of type I error by only retaining edges judged statistically significant may prevent artefactual group differences in "higher order" topological measures. This is relevant whether networks are thresholded to variable edge density using a fixed probability threshold, or whether fixed-density analyses are conducted using only subsets of participants whose edges are all statistically significant at a given density. Such methods are potentially applicable even if edge probabilities are directly related to edge weights: for example by being derived directly from standard correlations, across participants in structural networks or between BOLD time series with the assumption of nominal $df$ in functional networks. However, in such cases the type I error will be inflated, as shown in Patel et al. (2014).

Finally, the bootstrap resampling methods applied in Part II are applicable to any network constructed using pair-wise regional associations, including time series used to derive functional networks - if suitably extended to take into account the dependence between consecutive time-points in the time series (Kreiss and Lahiri, 2012).

### 7.3.2 Sliding window methods

The sliding window method applied in Part II is widely applicable. The simplest extension is to the study of structural network changes across different or wider age-ranges, including the entire life-span (Zuo et al., 2017).

However, more importantly, the independent variable (in this case, age) can potentially be replaced by any other continuous variable, including for example cognitive or clinical scores. This would enable extension of previous studies (e.g.: He et al., 2008; Yao et al., 2010; Collin et al., 2013) to study changes in structural network architecture as a function of cognitive function or disease in a continuous manner. However, such applications are conditional on suitable distribution of the independent variable, which should ideally be relatively uniform. Still, should it not appear uniform, potential relationships between sliding-window results and distribution of the underlying independent variable can be explored using methods presented in Chapter 5.

Finally, perhaps most broadly, the sliding window method is generalisable to any other type of network constructed using associations (such as correlations). One relevant example are networks of relationships between psychopathological symptoms (Borsboom and Cramer, 2013). Again, windows may be slid over age, or any other suitably distributed variable.
7.3.3 Characterisation of unthresholded networks

Some of the methods applied in Part III are broadly relevant. One example includes characterisation of changes in network weight at each percentile of the edge weight distribution. Instead of fitting models to understand changes with age, relationships of percentile-specific edge weights to other continuous variables or differences in weights between diagnostic groups across percentiles could be studied.

Similarly, the extraction of edge-wise effect sizes can be extended to other scenarios, such as the study of changes in disease. As in Chapter 6, these may be used to extract communities of nodes showing similar effects. For example, if applied to compare groups of healthy controls and patients, such analysis may yield "disease modules" of brain regions affected in a similar manner by the pathology.

As noted elsewhere in this thesis, other methods exist for the characterisation of fully weighted, unthresholded networks (Rubinov and Sporns, 2011). Together with the methods discussed above, these can yield summary measures of network architecture without the need to assume that certain edges are more important than others, as implied by thresholding.

Availability of data and code

To facilitate translation and extension of the methods presented herein to other contexts and to enable application of alternative methods to the underlying data, I have released analysis code and processed data related to Parts I and II of this thesis. Details are provided in Appendix B.

7.4 Recommendations

Below are guideline suggestions relating to construction and analysis of correlation-derived structural and functional brain networks. Although these recommendations do not focus on denoising or other pre- or post-processing steps, these should be carefully considered. Moreover, while these guidelines relate to Pearson’s correlation as a measure of association between regional time series, many apply to other methods of connectivity estimation as well.

Prior to the application of any potential threshold, the full weight distributions should be characterised. Most importantly, effects of mean connectivity, such as group differences or effects of age (depending on the question of interest of the study) should be well understood. Beyond group effects on the mean edge weight or higher moments, changes in individual percentiles of the distribution can be studied. Additionally, the application of methods for the
characterisation of fully weighted, unthresholded networks should be considered, including for example methods presented in Rubinov and Sporns (2011), or applied in Chapter 6.

If thresholding, I advise doing so according to some criterion of connection ‘likelihood’, rather than connection weights (e.g.: correlations). The probabilistic methods presented herein are an example of such criteria, for the specific cases of fMRI correlation networks (Part I) and structural covariance (correlation) networks (Part II). Furthermore, if studying networks which include negatively weighted edges, be aware of the potential effects on topological organisation of including these during thresholding. Generally, the choice of threshold should ideally be principled. Probabilistic thresholding lends itself well to a principled choice of threshold, based on statistical significance. In the present thesis, edges were retained when presenting $P_{FDR} < 0.01$. Still, it is worth verifying that results are not exclusive to a single threshold.

In networks thresholded to variable edge density (e.g.: by fixed $P$ value, such as $P_{FDR} < 0.01$ used herein), “higher-order” graph theoretical measures (such as efficiency) will retain a dependence on edge density (van Wijk et al., 2010). In this case, it may be preferable to focus on simpler graph measures such as edge density itself, or the architecture of connected components. For density-based thresholding, I would advise identifying what proportion of subjects contain significant or non-noisy edges at each edge density. At lower edge densities, a greater proportion of connectomes will contain only significant edges. Further, if wishing to enforce node-connectedness, the minimum-spanning tree method can potentially be applied (Alexander-Bloch et al., 2010); however, this risks the inclusion of a large proportion of false-positive edges, particularly at low edge densities. Therefore, the impact of such steps should be investigated.

Finally, it is worth investigating the relationship between any graph-theoretical measure used and mean connectivity. Previous work indicates that this might be weaker at lower edge densities, and suggests approaches to control for potential group effects of average edge weight in case-control comparisons (van den Heuvel et al., 2017). Investigation of such relationships should ensure that reported effects in graph-theoretical measures are not driven by potential underlying effects of mean connectivity.

**7.5 Future directions**

Beyond the application of methods presented in this thesis to other datasets, discussed in sections 7.2 and 7.3 above, the methods themselves could be extended.

For example, voxelwise estimates of effective $df$ following denoising of BOLD signal could be taken into account already while computing the association between regional
neurophysiological time series, enabling the analysis of unthresholded graphs adjusted for loss of $df$ and associated detection power.

Moreover, the sliding window method developed in Part II can be extended in numerous ways. As one limitation of structural correlation networks is their group nature, efforts have been made to derive measures of individual-subject contributions to structural network architecture using leave-one-out approaches (Saggar et al., 2015). Such methods could be extended to the sliding window method, where advantage could be taken of the fact that each participant contributes to several consecutive windows. Potentially, mixed-effect models could even be fitted to such data, akin to those fitted to longitudinal data. More generally, extension of the structural network paradigm to take into account longitudinal data would be beneficial, as only longitudinal data can provide us with a understanding of inter-individual differences in adolescent maturational trajectories (Foulkes and Blakemore, 2018).

Furthermore, extraction of edge-wise modules of effect size (as applied in Part III to obtain maturational communities) should be extended to include appropriate statistical tests of the significance of identified communities. For example, edge-wise methods to detect "clusters" of edges showing between-group differences have previously been developed (Zalesky et al., 2010), where significance of cluster sizes was evaluated using permutations.

Similarly, I have reported multiple spatial relationships between cortical maps, evaluated using Spearman’s correlations. However, the statistical significance of these relationships assumes independence of data points, which does not apply in this case. In Chapter 4, I have applied spatial permutation methods to deal with these effects, which have previously been applied in vertex-wise analyses (Alexander-Bloch et al., 2013b; Vandekar et al., 2015). However, as current versions of these tests rely on rotations of spherical projections of the cortical surface, they only apply to comparisons of cortical regions. Our consideration of subcortical regions in Parts I and III precluded the use of these sophisticated permutation tests. Thus, further formalisation of these tests (Alexander-Bloch et al., 2017) should focus on extending their applicability to subcortical areas.

Finally, more work is necessary to further map the relationships between steps and variables involved in network construction and the resulting topology. This will improve interpretability of network measures and ensure they capture true “higher order” effects, maximally independent of preceding steps. Accordingly, further work is required on development of suitable null models to control for confounds. Such endeavors will help fulfill a fundamental premise of (brain) network analysis: that interconnected patterns of nodes and edges within the network capture emergent properties; or in simpler terms, that the network is more than the sum of its parts.
REFERENCES


References


References

Greene, D. J., Laumann, T. O., Dubis, J. W., Ihnen, S. K., Neta, M., Power, J. D., Jr, J. R. P.,
of Functional Connections between the Basal Ganglia and Cerebral Cortex. Journal of

(2015). Characterizing the connectome in schizophrenia with diffusion spectrum imaging.


Gu, S., Satterthwaite, T. D., Medaglia, J. D., Yang, M., Gur, R. E., Gur, R. C., and Bassett,
D. S. (2015). Emergence of system roles in normative neurodevelopment. Proceedings of
the National Academy of Sciences, 112(44):13681–13686.

Hafkemeijer, A., Altmann-Schneider, I., de Craen, A. J. M., Slagboom, P. E., van der Grond,


Hagmann, P., Cammoun, L., Gigandet, X., Meuli, R., Honey, C. J., Wedeen, V. J., and Sporns,

Understanding Diffusion MR Imaging Techniques: From Scalar Diffusion-weighted
Imaging to Diffusion Tensor Imaging and Beyond. RadioGraphics, 26(suppl_1):S205–
S223.

ONE, 2(7):e597.

Hagmann, P., Sporns, O., Madan, N., Cammoun, L., Pienaar, R., Wedeen, V. J., Meuli,
connectivity in the late developing human brain. Proceedings of the National Academy of

Hajnal, J. V., Myers, R., Oatridge, A., Schwieso, J. E., Young, I. R., and Bydder, G. M.
Magnetic Resonance in Medicine, 31(3):283–291.

Hammelrath, L., Skocic, S., Khmelinskii, A., Hess, A., van der Knaap, N., Staring, M.,

Hawrylycz, M. J., Lein, E. S., Guillozet-Bongaarts, A. L., Shen, E. H., Ng, L., Miller,
An anatomically comprehensive atlas of the adult human brain transcriptome. Nature,


References


References


Appendix A

Overview of the Literature on Adolescent Brain Network Development
<table>
<thead>
<tr>
<th>Paper</th>
<th>Age (y)</th>
<th># Sub.</th>
<th>Nodes</th>
<th>Morph. feat.</th>
<th>Edges</th>
<th>Thresh.</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zielinski PNAS 2010</td>
<td>4.8-18.3</td>
<td>300</td>
<td>voxels</td>
<td>GM volume</td>
<td>ICA</td>
<td>P &lt; 0.001</td>
<td>FWE-corr.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• primary networks (+ DMN): early increases in extent, later pruning</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• higher-order networks: monotonic expansion in extent</td>
</tr>
<tr>
<td>Khundr. Cer. Cor. 2013</td>
<td>4.9-18</td>
<td>203</td>
<td>78</td>
<td>CT</td>
<td>Pears.</td>
<td>binary</td>
<td></td>
</tr>
</tbody>
</table>
|                       |         |        |       |              | r     | 5-25%      | • init. decr. in clustering (eff
loc & Q), incr. in glob. eff. (later reversed)                                                        |
|                       |         |        |       |              |       |            | • efficiency: paralimbic & association increase, sensorimot. decrease                           |
| Alex.-Bloch J. Neurosci. 2013 | 9-22    | 108    | 360   | Δ CT (long. data) | Pears. | binary     |                                                                                               |
|                       |         |        |       |              | r     | 1-30%      | • networks of cross-corr. of rates of thinning overlap                                               |
|                       |         |        |       |              |       |            | • static structural correlation (& functional) networks                                            |
|                       |         |        |       |              |       |            | • synchronised maturation ⇒ structural correlation?                                             |
| Moura Br. Im. Beh. 2016| 7-14    | 249    | 20    | • CT         | Pears. | none       |                                                                                               |
|                       |         |        |       |              | r     |            | • similar covariance trajectories of GM and WM                                                 |
|                       |         |        |       |              |       |            | • decreased covariance ("structural decoupling") in 9-11 age-group                              |
|                       |         |        |       |              |       |            | particulary involving forceps minor and connecting regions                                       |
| Khundr. Cer. Cor. 2017| 8.3-18.8| 141    | 78    | vertices     | matur
= cos similarity btw long. CT traj. | binary | 10-30% |                                                                                               |
|                       |         |        |       |              |       |            | • matur
= coupling overlaps static structural correlation                                            |
|                       |         |        |       |              |       |            | • developmental changes in coupling within DMN                                                  |
|                       |         |        |       |              |       |            | align with changes in struct. and funct. connectivity                                            |

Table A.1 Summary of adolescent development of structural correlation networks. Only studies whose age-range presents overlap with the NSPN study of adolescent development (ages 14-24 years) were selected; studies on "lifespan" development whose age-range extends up to old age were not included.
<table>
<thead>
<tr>
<th>Paper</th>
<th>Age (y)</th>
<th># Sub.</th>
<th>Motion *</th>
<th>GSR</th>
<th>Nodes</th>
<th>Edges</th>
<th>Thresh.</th>
<th>Results</th>
</tr>
</thead>
</table>
| Fair PNAS 2007   | 7-31    | 139    | motion match | yes  | 39    | Pears. r | r ≥ 0.1  | - sub-networks segregate with age  
|                  |         |        |          |      | 39    |        |         | - long edges increase in strength, short edges decrease                  |
| Fair PLoS CB 2009| 7-31    | 210    | none     | yes  | 34    | Pears. r | r ≥ 0.1  | - shift from 'local' to 'distributed' communities  
|                  |         |        |          |      | 34    |        |         | - community assignment changes; modularity stable  
|                  |         |        |          |      | 34    |        |         | - path length, clustering, small-worldness stable                        |
| Supekarn PLoS CB 2009| 7-22    | 45     | none     | no   | 90    | wav. corr. | binary 1-99% | - path length (λ), efficiency, clustering (γ), SWI σ stable  
|                  |         |        |          |      | 90    |        |         | - paralimbic weights incr., subcortical weights decr.                   |
|                  |         |        |          |      | 90    |        |         | - long edges increase, short edges decrease                            |
| Stevens HBM 2009 | 12-30   | 100    | none     | no   | voxels | Grang. caus. | N/A     | - linear (or log) decrease in causal interactions, esp. betw. lateral prefrontal-parietal circuits and DMN |
| Dosenb. Science 2010 | 7-30   | 238+195| none     | yes  | 160   | Pears. r | none    | - strengthening edges longer than weakening edges  
|                  |         |        |          |      | 160   |        |         | - within-module edges strengthen, betw.-module weaken                  |
|                  |         |        |          |      | 160   |        |         | - MVPA explains 55% variance of changes with age                        |
| Anderson Brain Con. 2011 | 7-35 | 1278   | none     | no   | 7266  | Pears. r | none    | - connectivity decrease betw. attention control and DMN  
|                  |         |        |          |      | 7266  |        |         | - DMN "boundaries sharpen" (conn. within incr.)  
|                  |         |        |          |      | 7266  |        |         | - slight decr.in connectivity within frontoparietal ROIs               |
| Satterthw. NeuroIm. 2013 | 8-22  | 780    | motion match | yes  | 264   | Pears. r | none    | - motion inflates effects of age & distance-dependence  
|                  |         |        |          |      | 264   |        |         | - motion dampens incr. in intra-modular connectivity                   |
|                  |         |        |          |      | 264   |        |         | - + large N needed to detect changes in conn. w/ age                   |
| Hwang Cer. Cor. 2013 | 10-20| 99     | time despike | no   | voxels | Pears. r | mult. wei. + bin. | - hub architecture stable  
<p>|                  |         |        |          |      | 160   |        |         | - early incr. betw. frontal hubs &amp; cort. + subc. non-hubs             |
|                  |         |        |          |      | 160   |        |         | - late incr. betw. cerebellar hubs &amp; cortical non-hubs                |</p>
<table>
<thead>
<tr>
<th>Paper</th>
<th>Age (y)</th>
<th># Sub.</th>
<th>Motion</th>
<th>GSR</th>
<th>Nodes</th>
<th>Edges</th>
<th>Thresh.</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu PLoS One</td>
<td>5.7-18.4</td>
<td>60</td>
<td>none</td>
<td>yes</td>
<td>90</td>
<td></td>
<td>Pears. r</td>
<td>binary 20-35% · path length (PL, ( \lambda )), efficiency, clust. (cc) stable</td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>· clustering (( \gamma )), SWI ( \sigma ), modularity Q increase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>· nodal changes in degree, efficiency and betw. cent.</td>
</tr>
<tr>
<td>Grayson PLoS One 2014</td>
<td>7-35</td>
<td>21</td>
<td>scrub.</td>
<td>yes</td>
<td>219</td>
<td></td>
<td>Pears. r</td>
<td>binary 1-10% · rich club strengthening (rich ( \rightarrow ) richer &amp; poor ( \rightarrow ) rich)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>· strengthening edges: somatosensory, insula (NBS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ structural (DWI) rich club organisation stable</td>
</tr>
<tr>
<td>Satterthw. PNAS 2014 *</td>
<td>8-22</td>
<td>922</td>
<td>covaried</td>
<td>N/A</td>
<td>voxels</td>
<td>Pears. r</td>
<td>N/A</td>
<td>· perfusion decr. nonlinear (min. ( \sim 17y )), esp. assoc. cortex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ trajectory diff. betw. females (decr. U) and males ( \sim \lin. )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(sex differences appear only mid-puberty)</td>
</tr>
<tr>
<td>Gu PNAS 2015</td>
<td>8-22</td>
<td>780</td>
<td>motion regress. from mat.</td>
<td>no, but mat. norm. by ( \mu )</td>
<td>264</td>
<td>Pears. r</td>
<td>none</td>
<td>· variance of module size &amp; similarity to adult modules incr.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>· DMN: within + between-module strength increases</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>· hi.-order, subc./cerebell.: w/in + betw.-mod. strength decr.</td>
</tr>
<tr>
<td>Marek PLoS CB 2015</td>
<td>10-26</td>
<td>192</td>
<td>wavelet despike + scrub.</td>
<td>no</td>
<td>264</td>
<td>Pears. r</td>
<td>binary 1-25% · module organization stable</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>· cing.-operc./salience part. coeff. incr. (( \rightarrow ) inhibitory control)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>· no distance-dependent change of strength</td>
</tr>
</tbody>
</table>

Table A.2 **Summary of adolescent development of whole-brain resting-state functional networks.** Only studies whose age-range presents overlap with the NSPN study of adolescent development (ages 14-24 years) were selected; studies on "lifespan" development whose age-range extends up to old age were not included. * Methods for motion correction listed in the "Motion" column do not include linear regression of motion parameters and their derivatives (which was applied by most studies). * The study by Satterthaite et al. (2014) (listed as Satterthw. PNAS 2014) is an arterial spin labeling (ASL) study.
Table A.3 **Summary of adolescent development of subcortical resting-state functional networks.** Only studies whose age-range presents substantial overlap with the NSPN study of adolescent development (ages 14-24 years) were selected; studies on "lifespan" development whose age-range extends up to old age were not included. * Methods for motion correction listed in the "Motion" column do not include linear regression of motion parameters and their derivatives (which was applied by most studies).

<table>
<thead>
<tr>
<th>Paper</th>
<th>Age (y)</th>
<th># Sub.</th>
<th>Motion *</th>
<th>GSR</th>
<th>Edges</th>
<th>hip</th>
<th>amy</th>
<th>die</th>
<th>pal</th>
<th>put</th>
<th>acc</th>
<th>cau</th>
<th>thal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qin PNAS 2012</td>
<td>7-22</td>
<td>87</td>
<td>none</td>
<td>yes</td>
<td>mult. regress&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑ para+limb. ass&lt;sup&gt;a&lt;/sup&gt;</td>
<td>vmPFC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gabard-D. NeuroIm. 2014</td>
<td>4-23</td>
<td>58</td>
<td>scrub. + FD reg.</td>
<td>yes</td>
<td>mult. regress&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑ mPFC</td>
<td>↓ ins. / STS</td>
<td>parah. / PCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greene J. Neurosci. 2014</td>
<td>7-31</td>
<td>180</td>
<td>scrub. no</td>
<td>partial corr.</td>
<td></td>
<td>↓ somatomotor face system (BG: pal. + put.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alarcon NeuroIm. 2015</td>
<td>10-16</td>
<td>122</td>
<td>scrub. yes</td>
<td>Pears. r</td>
<td>↓ par.-occip.</td>
<td>↓ mFC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fareri NeuroIm. 2015</td>
<td>4.5-23</td>
<td>66</td>
<td>scrub. + FD reg.</td>
<td>yes</td>
<td>mult. regress&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ventral striatum (put. + acc. + cau.)</td>
<td>↓ mPFC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sato NeuroIm. 2015</td>
<td>7-15</td>
<td>447</td>
<td>scrub. yes</td>
<td>Pears. r</td>
<td></td>
<td>↓ e-vec. cent.</td>
<td>↓ e-vec. cent.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Duij. NeuroIm. 2015</td>
<td>8-25</td>
<td>299</td>
<td>FD reg. yes</td>
<td>dual regress&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑ nucl. acc.</td>
<td>↑ dACC</td>
<td>hipp.</td>
<td>↑ dlPFC</td>
<td>↑ dlPFC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Methods for motion correction listed in the "Motion" column do not include linear regression of motion parameters and their derivatives (which was applied by most studies).
APPENDIX B

AVAILABILITY OF DATA AND CODE

Part I

Raw anatomical and functional scans were kindly made available by the Mind Research Network and University of New Mexico (http://fcon_1000.projects.nitrc.org/indi/retro/cobre.html). Code to perform wavelet despiking, estimation of effective $df$ and subsequent adjustment of $P$ values is available in the Brain Wavelet toolbox (v2.0), at www.brainwavelet.org. Processed data, including correlation and $df$-adjusted $P$ value matrices for all participants, has been uploaded to the Cambridge Data Repository (https://doi.org/10.17863/CAM.12827).

Matlab code used to conduct main analyses is available from my github: https://github.com/frantisekvasa/probabilistic_connectome (DOI: 10.5281/zenodo.847604).

Part II

Data used in this section has been uploaded to the Cambridge Data Repository (https://doi.org/10.17863/CAM.8856) and password protected. Participants within the NSPN study did not give informed consent for their questionnaire measures to be made publicly available, and it is possible that they could be identified from this data set. Access to the data will be made available to researchers with a reasonable request to NSPndata@medschl.cam.ac.uk.

The code used to conduct analyses is available from my github: https://github.com/frantisekvasa/structural_network_development (DOI: 10.5281/zenodo.528674).

Part III

Data analysed in this section, and code used for analysis, will be made available upon submission of the study for publication and deposition of the manuscript onto a pre-print server.
# Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOLD</td>
<td>blood oxygenation level dependent</td>
</tr>
<tr>
<td>CT</td>
<td>cortical thickness</td>
</tr>
<tr>
<td>df</td>
<td>degrees of freedom</td>
</tr>
<tr>
<td>DMN</td>
<td>default mode network</td>
</tr>
<tr>
<td>DWI</td>
<td>diffusion-weighted imaging</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalography</td>
</tr>
<tr>
<td>FC</td>
<td>functional connectivity</td>
</tr>
<tr>
<td>FD</td>
<td>frame-wise displacement</td>
</tr>
<tr>
<td>FDR</td>
<td>false discovery rate</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FWE(R)</td>
<td>family-wise error (rate)</td>
</tr>
<tr>
<td>GM</td>
<td>gray matter</td>
</tr>
<tr>
<td>GSR</td>
<td>global signal regression</td>
</tr>
<tr>
<td>ICA</td>
<td>independent component analysis</td>
</tr>
<tr>
<td>MEG</td>
<td>magnetoencephalography</td>
</tr>
<tr>
<td>ME-ICA</td>
<td>multi-echo independent component analysis</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MST</td>
<td>minimum spanning tree</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>MT</td>
<td>magnetization transfer</td>
</tr>
<tr>
<td>MWU</td>
<td>Mann-Whitney U (test)</td>
</tr>
<tr>
<td>NMI</td>
<td>normalised mutual information</td>
</tr>
<tr>
<td>NSPN</td>
<td>Neuroscience in Psychiatry Network</td>
</tr>
<tr>
<td>PCA</td>
<td>principal component analysis</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>sMRI</td>
<td>structural magnetic resonance imaging</td>
</tr>
<tr>
<td>WSR</td>
<td>Wilcoxon signed-rank (test)</td>
</tr>
<tr>
<td>WM</td>
<td>white matter</td>
</tr>
</tbody>
</table>
Mírou naděje v tomto hlubokém a silném smyslu není míra našeho rozveselení z dobrého běhu věcí a naší vůle investovat se do podniků, viditelně mířících k brzkému úspěchu, ale spíš míra naší schopnosti usilovat o něco proto, že to je dobré, a nikoli pouze proto, že to má zajištěno úspěch. Čím nepříznivější je situace, v níž svou naději osvědčujeme, tím hlubší tato naděje je. Naděje prostě není optimismus. Není to přesvědčení, že něco dobře dopadne, ale jistota, že má něco smysl – bez ohledu na to, jak to dopadne.

Václav Havel

Hope, in this deep and powerful sense, is not the same as joy that things are going well, or willingness to invest in enterprises that are obviously heading for success, but, rather, an ability to work for something that is good, not just because it stands a chance to succeed. The more unpropitious the situation in which we demonstrate hope, the deeper the hope is. Hope is definitely not the same thing as optimism. It is not the conviction that something will turn out well, but the certainty that something makes sense, regardless of how it turns out.

Translated by Paul Wilson