The impact of early institutional deprivation on brain structure and structural covariance in young adulthood

MacKes, Nuria Katharina

Awarding institution:
King's College London

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The impact of early institutional deprivation on brain structure and structural covariance in young adulthood

Nuria Katharina Mackes

Institute of Psychiatry, Psychology & Neuroscience
King's College London

A thesis submitted in part fulfilment for the degree of Doctor of Philosophy in Developmental Neuroscience

2019
Für meine Eltern und Manuel
ABSTRACT

Early institutional deprivation has been linked to a complex pattern of neurodevelopmental problems – aspects of which persist into adulthood, yet its impact on brain structure is currently unknown. This thesis investigates how early childhood institutional deprivation impacts adult brain structure and structural covariance. Structural magnetic resonance imaging data were acquired in a group of young adults who had been exposed to severe institutional deprivation during the Ceaușescu era in Romania before being adopted into nurturing families in early childhood. This prospective natural experiment is a powerful design because it allows the specific effects of early adversity on brain development and disorder to be studied independently of non-deprivation-related risks and familial genetic confounds which often limit the ability to draw causal inferences in non-prospective studies of maltreatment occurring within biological families. Sixty-seven Romanian adoptees, who had experienced between 3 and 41 months of institutional deprivation, were compared to 21 non-deprived UK adoptees. Data were analysed using surface-based morphometry methods.

Institutional deprivation in early childhood was associated with substantially smaller total brain volumes in young adulthood reflected in a dose-dependent negative association with deprivation duration. Above and beyond this global effect, institutionalisation was associated with a number of distinct regional changes. There was relatively larger cortical surface area, thickness and volume in the right inferior temporal cortex and relatively smaller surface area and volume in the right inferior frontal cortex following institutionalisation. Deprivation duration was positively associated with surface area and volume in right anterior cingulate cortex/medial orbitofrontal cortex. There were no effects of deprivation on subcortical structures including amygdala and hippocampus after correction for total brain volume.
When we tested for brain-behaviour relationships, a range of patterns were observed: Smaller brain volume was associated with increased risk of disinhibited social engagement symptoms and low IQ. In contrast, deprivation-related alterations of the right inferior frontal and temporal cortices were related to more effective proactive inhibition and better prospective memory and fewer ADHD symptoms, respectively - suggestive of neural compensation of either a latent (affecting underlying cognitive processes only) or fully manifest nature (reduced risk of disorder symptoms). There was also evidence of deprivation potentially creating latent vulnerability for future disorder with deprivation-related increases in right anterior cingulate volume associated with impaired empathic accuracy. In addition to these effects in regions of interest, on a whole-brain level, deprivation moderated the association between brain structure and symptoms of ADHD, ASD and IQ. Most striking was increased gyrification in those with high levels of ADHD symptoms who had experienced extended deprivation. This provides preliminary evidence that deprivation-related symptoms may have different neurobiological signatures compared to non-deprivation-related symptoms. Early institutional deprivation was also associated with alterations in structural covariance of cortical thickness and surface area with those having experienced more than 6 months deprivation demonstrating both increased as well as decreased correlation strengths between frontal and temporal brain regions with distinct changes for cortical thickness and surface area. These findings stress that deprivation may lead to brain structural alterations which in part reflect changes in coordinated brain network development, with the right fronto-temporal regions being most sensitive to deprivation.

Together, these findings provide compelling evidence that early institutional deprivation has strong neurobiological programming effects on brain development. It highlights how early adverse environments during sensitive periods of heightened
Abstract

neuroplasticity in the first few years of life can lead to persistent changes in brain structure that are present in young adulthood, more than 20 years after exposure to adversity has ended.
ACKNOWLEDGEMENTS

This PhD was an incredible journey and I could not have imagined how much I would learn and experience on the way. I loved every moment of it.

It takes a village to raise a scientist and there are many people I would like to thank who provided inspiration, mentoring, companionship, support and relief over the last three years.

First of all, I would like to thank my brilliant supervisors, Edmund Sonuga-Barke, Mitul Mehta and Graeme Fairchild. They were always there for me if I needed anything, from discussing fundamental research questions, advice on methods and statistics to cheering up (mostly combinations of these). They were a constant source of inspiration and made this journey a light-hearted one. If I learned anything, it is that we should not be afraid to ask big questions (even if or especially when we do not know the answer yet).

Special thanks go to the rest of the ERA and ERABIS team. In particular, I would like to thank Dennis Golm, who has been the best colleague one could have wished for, providing crucial cultural education (British bake off and Crufts), company and abode on Southampton days and entertainment during scanning sessions.

I would also like to thank Nicola Toschi, who not only offered me a desk in sunny Rome but also spent hours teaching me how to analyse in FreeSurfer in general and structural covariance in particular. Special thanks also go to Jana Kreppner who was my mentor (and briefly official supervisor) in Southampton and taught me a lot about teaching and the background of ERA. I would also like to thank Robert Kumsta, without whom I would not even have known about this study in the first place.
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This research would not have been possible without the continued commitment of our participants and their families, for which I am truly grateful. Thanks also to our radiographers and the dedicated staff at the Department of Neuroimaging.

My gratitude also goes to the Medical Research Council that funded this study.

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Schließlich, meine Eltern, danke, dass ihr stets an meine Träume glaubt, selbst wenn sie mich 300 Meilen (und eine Maßeinheit) von euch wegführen.
DECLARATION OF WORK

I joined the English and Romanian Adoptees Brain Imaging Study (ERABIS) in April 2015, when the study had already been designed (devised by Edmund Sonuga-Barke, Mitul Mehta and Graeme Fairchild) and data collection was underway. From April 2015 until February 2018, my colleagues Dennis Golm, Sagari Sarkar and I recruited participants and collected data for the study. Data collection included the neuropsychological testing and brain imaging sessions reported in this thesis. The calculation of polygenic scores for total intracranial volume was performed by Robert Kumsta and Linda Dieckmann. All measures acquired during previous follow-ups of the English and Romanian Adoptees Study, such as parent-rated symptom scores in young adulthood or weight at UK entry, were collected, prepared and made available by the English and Romanian Adoptees Study team. Neuropsychological data measuring proactive inhibition and prospective memory were prepared by Dennis Golm. Data from the empathic accuracy task were processed by me. I was solely responsible for quality checking and pre-processing of the structural brain imaging data acquired during ERABIS. All analyses presented in this thesis are my own work and were conceived and interpreted under the supervision of Edmund Sonuga-Barke, Mitul Mehta and Graeme Fairchild.
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<td>ADHD</td>
<td>attention-deficit/hyperactivity disorder</td>
</tr>
<tr>
<td>ASD</td>
<td>autism spectrum disorder</td>
</tr>
<tr>
<td>BEIP</td>
<td>Bucharest Early Intervention Project</td>
</tr>
<tr>
<td>CSA</td>
<td>cortical surface area</td>
</tr>
<tr>
<td>CT</td>
<td>cortical thickness</td>
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<tr>
<td>DSE</td>
<td>disinhibited social engagement</td>
</tr>
<tr>
<td>DTI</td>
<td>diffusion tensor imaging</td>
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<tr>
<td>ERA</td>
<td>English and Romanian Adoptees Study</td>
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<tr>
<td>ERABIS</td>
<td>English and Romanian Adoptees Brain Imaging Study</td>
</tr>
<tr>
<td>FA</td>
<td>fractional anisotropy</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>FWHM</td>
<td>full-width/half-max</td>
</tr>
<tr>
<td>HCP</td>
<td>Human Connectome Project</td>
</tr>
<tr>
<td>LoDep</td>
<td>low deprivation group combining UK adoptees and Romanian adoptees institutionalised for less than 6 months</td>
</tr>
<tr>
<td>MACACC</td>
<td>Mapping Anatomical Correlations Across Cerebral Cortex</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>n</td>
<td>sample size</td>
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<tr>
<td>PTSD</td>
<td>post-traumatic stress disorder</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>Rom</td>
<td>Romanian adoptees</td>
</tr>
<tr>
<td>Rom&lt;6</td>
<td>Romanian adoptees institutionalised for less than 6 months</td>
</tr>
<tr>
<td>Rom&gt;6</td>
<td>Romanian adoptees institutionalised for more than 6 months</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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SNP – single nucleotide polymorphism
TBV – total brain volume
UK – UK adoptees
CHAPTER 1

INTRODUCTION
1.1 **Highlights**

- Neuroplasticity refers to the brain’s ability to change in response to the environment.
- There are certain periods of development when neuroplasticity is greatest - termed sensitive or critical periods.
- The brain develops asynchronously and in a hierarchical manner with primary sensory areas generally developing early in life while the development of higher association cortices is more protracted.
- Early maltreatment impacts on brain development and is associated with long-term negative consequences on mental health.
- Prefrontal cortex and the hippocampus seem particularly vulnerable to the effects of early maltreatment.
- In humans it is hard to isolate the effects of adversity during critical/sensitive periods from other factors such as genetic risk or exposure to ongoing adversity.
- Studies of children adopted from depriving institutions such as orphanages can overcome some of the methodological limitations of studies of maltreatment in children living in their biological families.
1.2 **OVERVIEW**

This chapter will begin with an introduction to the concept of brain plasticity and critical and sensitive periods of development. It will review how the brain changes throughout development – which are most dramatic in the first two years after birth - to form complex, functionally organised and specialised interacting networks. Following this, it will review evidence for the impact of early maltreatment during this important period of brain development and the associated long-term negative effects on mental health and well-being. I will introduce initial insights obtained from animal models first, but then focus mainly on human studies of early maltreatment. This review will focus especially on studies of children experiencing early institutional deprivation because of their methodological advantages. I will then introduce the English and Romanian Adoptees Study, which is an example of such an institutional deprivation study and the basis of this PhD thesis. Following this I will state the aims and hypotheses of this thesis.
1.3 Neuroplasticity and Early Development

Neuroplasticity refers to the inherent ability of the central nervous system to dynamically change in response to stimulation from the environment, which enables normal development, learning and memory but also adaptation to and recovery from injury (Ismail, Fatemi, & Johnston, 2017). While neuroplasticity processes operate across the life-span (e.g. learning how to juggle in older age is associated with changes in grey matter volumes; Boyke, Driemeyer, Gaser, Büchel, & May, 2008), the brain’s potential for change is believed to be highest in the prenatal and early postnatal period (Dubois et al., 2014). In this context, the terms critical and sensitive periods have been coined, which refer to stages of development during which the brain is particularly malleable (Ismail et al., 2017).

Hubel and Wiesel (1964) were the first to describe a critical period of brain development. They found that if kittens were deprived of visual stimulation (by suturing one eye shut) during the first months after birth, electrophysical activity of neurons in the visual cortex was permanently altered and did not recover even after the eyelid was opened again. Later experiments showed that visual deprivation only altered the visual cortex if it occurred during the first three months of the cat’s life (Wiesel & Hubel, 1965).

A critical period can therefore be described as a stage during which a certain stimulation has to occur in order for normal brain development. After this period, the brain will not be able to adapt and change even in the presence of the stimulus (Hensch, 2005). The term of a sensitive period, on the other hand, suggests that the brain is particularly malleable during this period but the course of development can still be influenced during later stages, albeit less strongly (Ismail et al., 2017). Contemporary research in neuroscience mostly supports the less strong claim of sensitive periods, i.e. deprivation and injury can affect the brain at all stages of life.
and learning- and memory-related changes occur across the lifespan but there are certain periods in which brain areas are more prone to change (Voss, Thomas, Cisneros-Franco, & de Villers-Sidani, 2017). The evidence for such sensitive periods of brain development comes from multiple fields; for example it is easier for children to learn a second language compared to adolescents and adults (Newport, Bavelier, & Neville, 2001) and the optimal time for cochlear implantation is within the first four years of life (Kral & Sharma, 2012). There is also evidence that critical periods of plasticity can reopen in later life under certain circumstances. The visual cortex of adult cats, for example, reorganises following injury to one retina (Chino, Kaas, Smith, Langston, & Cheng, 1992).

Another important distinction with regard to neuroplasticity is the differentiation between experience-expectant and experience-adaptive programming (Rutter & O'Connor, 2004). The above described experiment on kittens by Hubel and Wiesel (1964, 1965) is an example for experience-expectant programming: The occurrence of a certain stimulus during a critical/sensitive period is expected and essential for normal cognitive and brain development. Deprivation of that stimulus would be considered an extraordinary environment and will lead to abnormal development (Greenough et al., 1987). Experience-adaptive programming, on the other hand, assumes a more flexible response pattern of the organism to its environment during sensitive periods. This concept assumes that the organism can be sculpted by the environment in a particular way that determines the further course of development depending on the stimulation during the sensitive period (Kolb & Gibb, 2014). From an evolutionary perspective, this mechanism would have evolved to prepare and adapt the organism for its likely later environment. The success of such an adaptation thereby depends on the similarity between the early environment during the sensitive period and later environments (Rutter & O’Connor, 2004). A
neurodevelopmental example for a case in which experience-adaptive programming has been proposed is attention-deficit/hyperactivity disorder (ADHD), which is marked by developmentally inappropriate hyperactivity, inattention and impulsivity. Pre- and postnatal exposure to stress might trigger an adaptation for dangerous environments, where symptoms of ADHD would be beneficial traits that allow to anticipate and quickly react to threats (Glover, 2011; Jensen et al., 1997). A related concept to experience-adaptive programming is latent vulnerability (McCrory & Viding, 2015), which states that changes in neurobiology in response to early maltreatment promote adaptation to these maltreating environments. However, in the long term these changes become maladaptive and increase vulnerability for developing psychopathology (McCrory & Viding, 2015).

The above examples also show why neuroplasticity has been described as a double-edged sword (Stevens & Neville, 2006). On the one hand, it allows learning, memory, adaptation, compensation and recovery from brain injuries. On the other hand, brain changes associated with experience-adaptive programming might be maladaptive in later life. In the case of experience-expectant programming, deprivation in early life could be associated with long-term negative consequences (McLaughlin, Sheridan, & Lambert, 2014). Furthermore, increased plasticity might leave the brain particularly vulnerable to insult during sensitive periods, increasing the risk of neural damage (Rutter & O’Connor, 2004). The focus of this thesis – the effects of early institutional deprivation on brain structure – provides an example of such neuroplasticity as a double-edged sword. Early deprivation has been associated with long-term negative effects on neuropsychiatric symptoms and well-being (Sonuga-Barke et al., 2017). This thesis explores brain structural alterations following institutional deprivation which might be underlying neurodevelopmental symptoms. Importantly, not everyone who experienced early deprivation presents
the same symptom profile in later development, but there is considerable variation within individuals over time and across individuals. It is therefore important to distinguish factors of resilience and compensation from factors of vulnerability and increased disorder risk when studying early neuroplasticity as a link between early adverse environment and later disorder. If brain alterations following early adverse experiences are associated with negative effects on later development, they could represent manifest disorder risk by directly explaining increased symptom rates (Perry, 2010). Brain alterations might also confer latent cognitive vulnerability as described by McCrory and Viding (2015). In this case, early adverse experiences alter or lead to adapted neurocognitive functioning which might indirectly increase risk of later disorder. On the other hand, it is possible that brain structural changes following early adverse environments might be associated with positive or at least not negative neurodevelopmental and neuropsychological outcomes in later development. These brain alterations might indicate resilience, either because of pre-existing factors such as a certain genetic profile or because of compensatory processes during or after exposure to adversity which promote resilience. As with vulnerability, compensatory changes might be manifest or latent.

Before evaluating existing research on the effects of early maltreatment in general and deprivation in particular on the brain, I will first provide an overview on brain development, particularly focusing on the most critical first two years after birth.

1.3.1 Imaging early development

It has been extremely challenging to study the development of the brain in early postnatal life. In the past, most studies of children’s brains relied on (thankfully) rare post-mortem examinations, which provided detailed insights regarding macro- and
microstructural changes but were limited by sample size and cross-sectional
designs (Gilmore et al., 2018). Animal models have also been employed, however,
human development is unique in its protracted nature, with some regions not being
fully developed before the mid-twenties (Dubois et al., 2014).

The introduction of in vivo imaging techniques, such as magnetic resonance
imaging (MRI; the focus in this thesis) and electroencephalogram, have enabled
researchers to examine the human brain non-invasively. MRI scanning of new-
borns and toddlers is associated with challenges (these include, e.g. motion
artefacts, changing magnetic properties of different brain tissues throughout
development) and only allow limited inferences on the cellular level. Nonetheless,
they have been able to provide valuable insights into the first years of human brain
development (for a more extensive review see Gilmore, Knickmeyer, & Gao, 2018).

The brain develops rapidly in the first two years of life. At birth, the brain’s size is
on average about 35% the size of that of an adult (Gilmore et al., 2007) and
reaches about 80% 2 years after birth (Knickmeyer et al., 2008). Grey and white
matter thereby follow different developmental trajectories.
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1.3.2 Grey matter

1.3.2.1 Cortex

The cortex develops rapidly during the first two years after birth. In one longitudinal study, cortical thickness (CT) increased by an average of 36% while cortical surface area (CSA) increased by 115% between birth and two years of age (Lyall et al., 2015, Figure 1.2). Both numbers dwarf the changes in CT and CSA seen in later years. By age 2, the brain has reached 97% of adult values for CT and 69% for CSA (Lyall et al., 2015). This implies that CT is more established at birth than CSA. Cortical gyrification develops even faster than the other two measures and occurs mostly prenatally, so there are limited changes (a 16% increase) during the first year of life (Li, Cao, et al., 2015).

Figure 1.1: Longitudinal T1 and T2 brain scans at 2 weeks, one year and two years of age. Reprinted from Gilmore et al., longitudinal development of cortical and subcortical gray matter from birth to 2 years, Cerebral Cortex, 2012, 22 (11), p 2479, by permission of Oxford University Press.
Figure 1.2: Overview of development of different brain measures. Cortical grey matter increases dramatically within the first two years of life followed by a slower increase and reductions in the second decade. Cortical white matter increases are more attenuated and continue throughout the first two decades. Fractional anisotropy (FA) increases are most pronounced in the first two years and increase slowly afterwards. Cortical thickness increases substantially within the first two years, followed by thinning. Cortical surface area expands rapidly in the first two years and more slowly afterwards. Adapted by permission from Springer Nature, Nature Reviews Neuroscience, Imaging structural and functional brain development in early childhood, Gilmore, J.H., Knickmeyer, R.C., Gao, W., 2018.

CT and CSA growth rates are highly heterogenous across the cortex (Lyall et al., 2015). CT and SCA both generally follow hierarchical developmental trajectories with primary and secondary sensory cortices showing the slowest growth (and being more established at birth) while regions implicated in speech, higher order association cortices and insula and cingulate showing the fastest growth rates (Lyall et al., 2015).

By the age of 2 years, most brain regions have reached peak CT, which then starts to gradually decrease (with some regions such as the anterior cingulate cortex showing thinning even earlier; Lyall et al., 2015) while CSA continues to expand until 8 – 12 years when it starts to decrease continuously (Gilmore et al., 2018; Wierenga, Langen, Oranje, & Durston, 2014).
What are the cellular mechanisms that lead to such dramatic increases in size of the cortex? While most neurons form prenatally, there is evidence for neurogenesis after birth in frontal regions of the brain (Sanai et al., 2011), which could contribute to local CT and CSA expansion. As Figure 1.3 shows, the first two years of life are marked by abundant synaptogenesis, with synapse numbers peaking earlier in sensory areas compared to frontal association areas (consistent with growth trajectories in CT and CSA; Huttenlocher & Dabholkar, 1997). During early childhood synaptic connectivity by far exceeds that of adults and this peak is followed by synaptic pruning (reflected by decreases in CT; Lyall et al., 2015; van Dyck & Morrow, 2017). Likewise, dendritic arborization contributes to higher complexity of neurons and synaptic spine density reaches its maximum at around 2 – 5 years (Petanjek et al., 2011). Gliogenesis is also a major contributor to the rapid increase in brain volume (van Dyck & Morrow, 2017). Again, these processes might peak at different times depending on the cortical region and even layer within the region, but they all show their most drastic increases in the first two years of life.

**Figure 1.3:** Connectivity patterns of cortical neurons increase quickly after birth. There is abundant synaptogenesis, dendritic arborization and increasing synaptic spine density. Adapted with permission from Springer Nature, Nature Reviews Neuroscience, Imaging structural and functional brain development in early childhood, Gilmore, J.H., Knickmeyer, R.C., Gao, W., 2018.
1.3.2.2 Subcortical structures

Subcortical grey matter structures follow similar dramatic increases in volume in the first years of life as cortical grey matter. In the first year, most subcortical structures increase by about 105% in volume with the exception of the hippocampus which increases in volume by only about 85% (Gilmore et al., 2012). This is followed by more variable increases in subcortical volumes ranging from around 8% in the putamen to around 18.5% in pallidum and hippocampus in the second year. The cellular processes involved in the development of subcortical structures are likely similar ones compared to those implicated in cortical grey matter.

1.3.3 White matter

In contrast to grey matter, white matter morphology is largely established at birth and then develops more slowly postnatally. White matter volumes increase by 11% in the first year and a further 19% in the second year (Figure 1.2; Knickmeyer et al., 2008). Nonetheless the maturation of white matter tracts, which connect cortical regions and neurons with each other, increases dramatically as measured by diffusion tensor imaging (DTI) methods (as reviewed by Dubois et al. 2014). The main cellular process by which this maturation occurs is myelination (i.e. myelin formation around axons). DTI studies use measures such as fractional anisotropy (FA) which correlates with microstructural properties of white matter such as myelination but also fibre diameter, fibre density and membrane permeability (Jones, Knösche, & Turner, 2013). These studies show that white matter maturation follows the same hierarchical principles in its progression as grey matter maturation: It occurs earlier in (1) sensory pathways than motor ones, and (2) projection fibres than association fibres. It also occurs faster in proximal pathways than distal ones (Dubois et al., 2014).
1.3.4 Grey matter networks

Adult brain structure is organized as a network, with properties such as CT of different brain regions often covarying across individuals (the first studies showing this were by He, Chen, & Evans, 2007; and Lerch et al., 2006). Structural covariance studies investigate how properties such as CT and CSA of different brain regions are correlated. Graph theory measures can be employed to study such networks, where brain regions are defined as nodes and their connections (for example correlation of CT) as edges (Bullmore & Sporns, 2009). Strikingly, the neonate brain already shows similar network properties as the adult brain: It exhibits small-world properties (with many short- and few long-range connections) and is organized into functional modules (which show many within-module connections but few outside-module connections) (Fan, Shi, et al., 2011). Within the first two years after birth, we see increases in both modularity (the segregation of connections into modules) and global efficiency (the shortest distance between nodes) (Fan, Shi, et al., 2011). This implies a successful integration and specialisation of brain regions across the cortex via so called hub-nodes that efficiently connect otherwise segregated modules (Baum et al., 2017). By age 5, sensorimotor networks are already well-developed, while association networks follow a more protracted development throughout childhood and adolescence (Khundrakpam et al., 2013; Zielinski, Gennatas, Zhou, & Seeley, 2010).

While the biological meaning of structural covariance remains unclear, it is thought to result in part from maturational coupling: Brain regions that develop together tend to be more similar in CT values and function. Between 9 and 22 years, longitudinal maturational covariance maps (measuring maturational rates of CT) are similar to cross-sectional structural covariance maps of CT (Alexander-Bloch, Raznahan, Bullmore, & Giedd, 2013; Khundrakpam et al., 2017). However,
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during the first two years after birth, maturational maps do not show a close relation
to structural covariance but display a high overlap with functional connectivity, as
measured using temporal synchrony of brain activity (Geng et al., 2017). This
suggests that functional networks develop prior to structural grey matter networks
and early functional connectivity patterns might guide the establishment of later
structural covariance patterns (Geng et al., 2017). This is supported by a partial
overlap between structural covariance and functional connectivity in adults
(Alexander-Bloch, Raznahan, et al., 2013; Segall et al., 2012). Structural covariance
networks also show partial convergence with white matter axonal connections as
measured by DTI (Gong, He, Chen, & Evans, 2012). The protracted development of
white matter volume increases and the underlying cellular mechanism of axonal
myelination are thought to be contributors to the slower establishment of structural
covariance networks. However further research is needed to establish the temporal
sequence and the mechanisms involved.

It should be noted that all of the above studies investigate either cortical volume
(Fan, Shi, et al., 2011) or CT (Alexander-Bloch, Raznahan, et al., 2013; Geng et al.,
2017; Khundrakpam et al., 2017; Khundrakpam et al., 2013). Gyrification networks
develop similarly compared to CT networks between 3 and 20 years (Nie, Li, &
Shen, 2013). I was unable to identify any studies on the development of CSA
covariance. CSA and CT covariance maps show minimal overlap in adulthood
(Sanabria-Diaz et al., 2010), which may be expected because these measures have
distinct developmental trajectories (Lyall et al., 2015; Wierenga et al., 2014) and are
genetically distinct (Panizzon, Fennema-Notestine, Eyler, Jernigan, Prom-Wormley,
Neale, Jacobson, Lyons, Grant, & Franz, 2009) suggesting that their covariance
maps will also follow different developmental trajectories.
1.3.5 Link to behaviour

One aim of developmental neuroscience research is ultimately to link brain changes to behaviour, to predict risk of developing neuropsychiatric diseases and - in the long-term - identify target mechanisms for early intervention (Gilmore et al., 2018; Sonuga-Barke, 2014). To date, it has proven difficult to identify such early structural biomarkers for later behaviour and symptoms. There is no single pathway between brain and behaviour, but rather a multifaceted interplay of brain regions will determine multifaceted changes in behaviour (Woo, Chang, Lindquist, & Wager, 2017). Mapping one on the other is especially difficult during a time of rapid and dynamic development, where brain-behaviour relationships are likely to be age-dependent (as seen in Fjell et al., 2012). Moreover, heterogeneity in neurodevelopmental research findings stems not only from variation within participants over time but symptom profiles and severity vary across individuals and are likely to represent distinct subgroups of underlying biological pathways (Lai, Lombardo, Chakrabarti, & Baron-Cohen, 2013). The employment of carefully designed, prospective studies will allow us to advance our understanding of the brain mechanisms involved in normal and pathological development and to model and predict individual trajectories of neurodevelopment.

There is emerging consensus that neurodevelopmental disorders such as autism spectrum disorder (ASD) manifest neurobiologically within the first few years after birth. To be potentially useful as structural biomarkers, brain differences should be apparent before the onset of symptoms (Yerys & Pennington, 2011). ASD research shows the most promising results for potential brain biomarkers (for review see Gao et al., 2019). For example, hyper-expansion of CSA between 6 and 12 months after birth predicted the diagnosis of autism in high-risk individuals when 24 months old (Hazlett et al., 2017). For ADHD, the ENIGMA-ADHD consortium has
reported modest accuracy in predicting ADHD diagnosis, with smaller total intracranial volume being the most important predictor followed by surface area of multiple cortical regions and subcortical volumes (Zhang-James et al., 2019). While this cross-sectional study only examined individuals who had already been diagnosed with ADHD (compared to healthy controls), a longitudinal study found that smaller brain volumes at birth, particularly volume in the right dorso-prefrontal cortex, predicted persistent ADHD symptoms in childhood in individuals born very preterm (Bora, Pritchard, Chen, Inder, & Woodward, 2014). These findings provide initial insights into potential brain structural biomarkers of neurodevelopmental disorders. But they also highlight that it would be informative to employ longitudinal study designs to investigate brain structural and neurodevelopmental trajectories rather than focusing on single time points (Ambrosino, Wierenga, de Zeeuw, Durston, & van Dijk, 2017).

1.3.6 Influence of genes and environment

The human brain and its development are shaped by a combination of genetic, epigenetic and environmental influences. Twin studies investigate the relative influence of genetic and environmental factors. These studies suggest that both grey and white matter volumes show relatively high heritability, with about 70 – 90% of the variance in adults explained by genetic factors (Baaré et al., 2001; Jansen, Mous, White, Posthuma, & Polderman, 2015; Kremen et al., 2010). In neonates, heritability of white matter is similarly high to that of adults (85%), while grey matter shows lower heritability of 56% (Gilmore et al., 2010), which then increases throughout childhood and adolescence (Giedd, Schmitt, & Neale, 2007). CT and CSA also show medium to high heritability, however their shared genetic variance is low, suggesting that they are influenced by distinct genetic factors (Panizzon,
Fennema-Notestine, Eyler, Jemigan, Prom-Wormley, Neale, Jacobson, Lyons, Grant, & Franz, 2009). In contrast to the high heritability of total grey and white matter volume, CT and CSA, heritability of CT in specific brain regions is very heterogenous with primary sensorimotor regions showing higher heritability in young childhood compared to later developing association areas (Lenroot et al., 2009). The heritability of CT in association areas increases in later childhood and adolescence (Lenroot et al., 2009; Schmitt et al., 2014).

While twin studies provide an elegant way to study genetic and environmental influences, they are limited in their ability to account for the role of certain types of influences on brain development. First, genes and environment might show interactive effects, which cannot be sufficiently explored in such designs. Studies specifically investigating gene x environment interactions have mostly focused on the moderating influence of candidate genes on environmental effects, but recently studies have started to calculate polygenic risk scores for certain psychiatric disorders based on multiple significant contributor genes derived from genome-wide analyses (Major Depressive Disorder Working Group of the Psychiatric Gwas Consortium et al., 2012). For example, polygenic risk scores for depression moderate the influence of ante-natal maternal depressive symptoms on right amygdala volume in infants (Qiu et al., 2017). Second, cases of disrupted experience-expectant programming due to extraordinary environments as described in the beginning of this chapter are most likely very uncommon in twin study designs. They might therefore be suitable for estimating the influence of variance in the normal environment but limited regarding extraordinary environments.

The effect of environment influences on brain development has also been investigated in observational studies that do not employ twin study designs. These studies show that brain development correlates with exposure to environmental
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stimuli, ranging from maternal behaviour and mood (Qiu et al., 2017), nutrition (Prado & Dewey, 2014), and socio-economic status (Amso & Lynn, 2017), through to traumatic experiences and early life stress (Lupien, McEwen, Gunnar, & Heim, 2009). The effects of early maltreatment on brain structure is an excellent example for this type of research and will be the focus for the rest of this review. Neuroplasticity as described earlier is the putative underlying mechanism of such environmental effects, whereby the brain’s developmental trajectory will be altered depending on the presence or absence of the stimulus. However, it is difficult to prove that neuroplastic changes in response to the adverse environment cause long-term changes in physiology - when these adversities are imposed by genetically related individuals in biological families, mostly due to familial confounding with genetic risk, as we will see in 1.4.5.

1.3.7 Interim conclusion

Brain development is asynchronous and hierarchical with grey and white matter developing earlier in primary sensory areas and showing more protracted development in higher association areas. The most rapid changes are observed in the first two years of life followed by more gradual changes thereafter. Brain development can be linked to behaviour and is strongly influenced by genetic as well as environmental factors. The impact of the environment thereby depends on sensitive periods, during which certain brain regions (and structural measures) are particularly malleable to change.
1.4 Early Maltreatment and the Brain

Early maltreatment, the experience of abuse and/or neglect in the first few years of life, is considered one of the strongest predictors for poor mental health and well-being across the life span (Green, McLaughlin, Berglund, & et al., 2010; McCrory, Gerin, & Viding, 2017). It has been associated with increased risk for multiple psychiatric disorders in childhood and adulthood (Gilbert et al., 2009; Green, McLaughlin, Berglund, & et al., 2010; Teicher & Samson, 2013). These include depression, generalized anxiety disorder (Gallo, Munhoz, Loret de Mola, & Murray, 2018) post-traumatic stress disorder (PTSD), psychotic experiences (Croft, Heron, Teufel, & et al., 2018), conversion disorder (Ludwig et al., 2018), conduct disorder (Braga, Gonçalves, Basto-Pereira, & Maia, 2017), substance use disorder (Halpern et al., 2018), ASD, ADHD (Sonuga-Barke et al., 2017), and disinhibited social engagement, (DSE; Kennedy et al., 2017). Beyond psychiatric diagnosis, individuals with a history of early maltreatment are more likely to show impaired cognitive functioning, with most pronounced effects in executive functions (Kavanaugh, Dupont-Frechette, Jerskey, & Holler, 2017). Physiological consequences have also been reported with higher rates of obesity (Li, Chassan, Bruer, Gower, & Shelton, 2015) and diabetes (Shield et al., 2016) found in maltreated individuals.

It has been hypothesized that these biopsychosocial effects of early maltreatment can be explained by disruptions and alterations in brain development (Perry, 2010; Teicher & Samson, 2016). Research on early maltreatment, therefore arguably provides the most potent example on how increased plasticity might leave the brain particularly vulnerable to hostile environments during the first years of life, altering brain developmental trajectories and long-term negative effects (McCrory et al., 2017). By examining the effects of early maltreatment on the brain, researchers
can gain a better understanding of the early neuroplastic processes involved and how to target these for intervention. I will review evidence for this hypothesis, by focusing first on animal models of early life stress and second on human observational studies. Finally, I will review evidence from adoption-based human studies specifically. The questions, I would like to answer with this review are as follows:

1. Does early maltreatment disrupt brain development in children, adolescents and adults?
2. Are some brain regions or structural properties more vulnerable than others?
3. How do these brain changes predict the emergence of later psychopathology?
4. How can we distinguish maltreatment- and psychopathology-related brain changes?
5. How can the effects of maltreatment be disentangled from confounding genetic and environmental risk factors?

1.4.1 “Getting under the skin”: animal studies of early life stress identify putative biological mechanisms

The discovery of glucocorticoid and mineralocorticoid receptors in the hippocampus (Reul & De Kloet, 1985) is considered the “gateway” by which research started advancing our understanding of how stress affects the brain (McEwen, Nasca, & Gray, 2015). Research has since expanded to consider other inter-connected regions such as the amygdala and the prefrontal cortex, all of which show a high density of glucocorticoid receptors (Morimoto, Morita, Ozawa, Yokoyama, & Kawata, 1996). Via these receptors the brain receives hormonal feedback from the hypothalamus-pituitary-adrenal (HPA) axis, the endocrine stress system of the body
The hippocampus in turn downregulates HPA axis activity (Strüber et al., 2014).

Animal models show that early life stress (in mice mostly induced by maternal separation, inadequate nesting material or observation of natural variations in maternal care) changes the “programming” of the HPA axis (Lupien et al., 2009) and has long lasting effects on brain development and behaviour (Sanchez, McCormack, & Howell, 2015). For example, early life stress has been associated with lower density of glucocorticoid receptors in the hippocampus which might be a mechanism for impaired HPA axis downregulation following early life stress (Liu et al., 1997). Accordingly, structural and functional alterations following early life stress are most prominent in brain regions associated with the HPA axis (Lupien et al., 2009). Stress thereby affects different structures in a different manner: While it leads to increased new spine formation and longer dendrites in the amygdala, which contributes to an increase in volume, the opposite pattern is found in the hippocampus and the prefrontal cortex, which decrease in volume due to dendritic atrophy, debranching and cell death (Monroy, Hernández-Torres, & Flores, 2010; Sapolsky, Krey, & McEwen, 1985; Vyas, Mitra, Rao, & Chattarji, 2002).

The timing of early life adversity is important, as postnatal days 4 – 14 in mice are usually marked by a stress hyporesponsive period, where stressors do not increase glucocorticoid secretion (Levine, 1994). This is thought to represent an evolutionary mechanism to protect the brain’s rapid development from heightened glucocorticoid levels (Lupien et al., 2009). However, the hypo-responsive period is mediated by maternal care and maternal separation and poor maternal care leads to an increased secretion of glucocorticoids even during this period (Lupien et al., 2009). This is an important potential mechanism by which early life stress in the
absence of maternal care can disrupt brain development and even more so during sensitive periods.

Of course, alterations in brain structure and function following early life stress also depend on additional influences. Biological embedding of early life adversity, the process by which initially transient homeostatic processes alter long-term physiology, has been described in neuroendocrine, immune, metabolic and microbiotic systems (Berens, Jensen, & Nelson, 2017).

Oxytocin, for example, is another prominent hormone that has been shown to be affected by early life stress, with fewer oxytocin receptors in the amygdala after maternal separation (Francis, Champagne, & Meaney, 2000). There is also emerging evidence that gut microbiota influence the stress response in early life (O’Mahony, Clarke, Dinan, & Cryan, 2017).

In conclusion, animal studies allow us to identify the mechanisms by which early adversity gets under the skin. These studies suggest that some brain regions, for example those that have a high density of glucocorticoid receptors, are more susceptible to early life stress.

1.4.2 Human brain imaging studies of early maltreatment

In recent years, multiple studies have attempted to examine the effects of childhood maltreatment on the brain. Research on this topic is highly heterogeneous and differs in several of the following ways. These variations are also linked to potential limitations of study designs which might restrict causal inferences on the effects of maltreatment, and which will be described in detail in 1.4.5.

1.4.2.1 Types and severity of maltreatment

Maltreatment research often differentiates between 5 types of childhood trauma: emotional abuse, physical abuse and sexual abuse as well as physical neglect and
emotional neglect (often measured and categorized using the self-report Childhood Trauma Questionnaire; CTQ; Bernstein et al., 1994). Different cut-offs derived from population statistics exist to classify severity of the different maltreatment types. Often, studies focus on a particular type of maltreatment. These studies will sometimes exclude participants with multiple forms of maltreatment, to be able to establish its unique effects, or include individuals with multiple types, as this is more reflective of the general population. Even though it has been suggested relatively early that different types of maltreatment exert different effects on brain development (Glaser, 2000), only recently, studies, reviews and meta-analyses have started to directly compare the effects of different types of maltreatment (e.g. Everaerd et al., 2015).

1.4.2.2 Recruitment from population-based versus high-risk groups

Some studies screen participants from the general population, while others target neighbourhoods that have a higher prevalence of maltreatment or vulnerable groups which are more likely to have experienced maltreatment such as foster children.

1.4.2.3 Age at which maltreatment was experienced

I will focus on studies, where maltreatment was experienced during early childhood (as opposed to later childhood or adolescence). However, as most studies rely on retrospective self-reports like the CTQ, the precise timing of these experiences is often difficult and is difficult to date before the age of 3 years. Moreover, many individuals who experience early maltreatment, continue to experience such adversities throughout their development making it difficult to identify sensitive periods of development during which the brain is particularly vulnerable to maltreatment (Dunn et al., 2011).
1.4.2.4 Age at which participants took part in the study

Most studies focus on the brain developmental effects in children and adolescents, even though a growing body of research has started to identify the neural correlates of early maltreatment in adulthood (e.g. Dannlowski et al., 2012).

1.4.2.5 Psychopathology

As discussed above, risk for psychopathology is substantially higher in individuals who experienced early maltreatment (Gilbert et al., 2009). Moreover, psychopathology following early maltreatment tends to show higher comorbidity, reduced treatment response and higher persistence, which is why it has been hypothesised that deprivation-related variants of these disorders might be neuroanatomically distinct (Teicher & Samson, 2013). Some studies have started addressing this question by comparing individuals with deprivation-related psychopathology such as psychosis (Sheffield, Williams, Woodward, & Heckers, 2013) and borderline personality disorder (Morandotti et al., 2013) to participants with the same diagnosis but without early maltreatment exposure. Another research question focuses on interindividual variability: What factors determine whether individuals exposed to early maltreatment develop subsequent psychopathology? Only few studies have specifically examined the concepts of resilience and vulnerability by comparing individuals with early maltreatment who either did or did not develop psychopathology (e.g. De Bellis et al., 2015; Morey, Haswell, Hooper, & De Bellis, 2015; Sun, Peverill, Swanson, McLaughlin, & Morey, 2018).

1.4.2.6 Prospective longitudinal vs retrospective cross-sectional

Many studies in this field are retrospective and cross-sectional. Prospective, longitudinal studies can overcome potential bias in self-report and recruitment and
therefore support causal inferences of the influence of early maltreatment on brain development.

For a comprehensive review of published studies examining the effects of childhood abuse and neglect on brain structure, see (Teicher & Samson, 2016), as well as (Bick & Nelson, 2016). Current meta-analyses and reviews on the neurobiology of childhood maltreatment show that some common brain areas and neural mechanisms can be identified, which seem to be sensitive to childhood maltreatment (Bick & Nelson, 2016; Lim, Radua, & Rubia, 2014; McCrory et al., 2017; Paquola, Bennett, & Lagopoulos, 2016; Teicher & Samson, 2016). The current literature review will mostly focus on findings derived from meta-analyses, as these use statistical methods to provide robust quantitative summaries of the existing literature.

1.4.3 Early maltreatment and brain structure

1.4.3.1 Total brain volume

Early structural magnetic resonance imaging (MRI) studies investigating children who experienced different forms of maltreatment found reductions in overall grey and white matter volume (Carrion et al., 2001; De Bellis et al., 2002). In a more recent study, childhood neglect was associated with reductions in total grey matter volume in patients with schizophrenia (Cancel et al., 2015). Most studies on early maltreatment have employed whole-brain or region of interest (ROI) approaches to identify specific brain regions that are affected, rather than examining total grey or white matter differences.
1.4.3.2 Grey matter

Cortex

The prefrontal cortex has been hypothesised to be particularly vulnerable to early maltreatment because of its protracted development (see 1.3.2.1), high density of glucocorticoid receptors (Morimoto et al., 1996) and postnatal neurogenesis (Sanai et al., 2011). Moreover, most higher order cognitive processes, such as executive functioning, heavily depend on the prefrontal cortex (Yuan & Raz, 2014), many of which have been shown to be impaired following maltreatment. Therefore, many studies of maltreatment have chosen prefrontal regions as ROIs (Teicher and Samson (2016); for an illustration of the functional divisions of the prefrontal cortex see Figure 1.4).

![Diagram of prefrontal cortex](image)

**Figure 1.4: Functional organisation of the prefrontal cortex.** A) presents a lateral view while B) shows ventral regions. dmPFC – dorsomedial prefrontal cortex; dIPFC – dorsolateral prefrontal cortex; vmPFC – ventromedial prefrontal cortex; vIPFC – ventrolateral prefrontal cortex; OFC – orbitofrontal cortex, ACC – anterior cingulate cortex. From Carlén, M. (2017), What constitutes the prefrontal cortex? *Science, 358*(6362), 478-482. Reprinted with permission from AAAS.

Indeed, the most consistent findings across studies are smaller volumes in one or more regions of the prefrontal cortex (Teicher & Samson, 2016). These include reduced volume associated with early maltreatment in the anterior cingulate cortex in adults (Cohen et al., 2006; Dannlowski et al., 2012; Sheffield et al., 2013; Thomaes, Dorrepaal, Draijer, Smit, & Veltman, 2010; Tomoda, Suzuki, et al., 2009), while results for adolescents are mixed (Hanson et al., 2012; Morey et al., 2015).
Reductions in orbitofrontal cortex volume were also reported in children (De Brito et al., 2013; Hanson et al., 2010) and adolescents (Edmiston et al., 2011) and ventromedial prefrontal cortex reductions were observed in young adults (Tomoda, Suzuki, et al., 2009).

Fewer studies have investigated morphometric measures other than volume and only a very limited number of studies have systematically studied more than one measure (Kelly et al., 2016; Kelly et al., 2013; Lim et al., 2018). In line with volumetric findings above, smaller CT of the anterior cingulate cortex has been reported in adults (Gupta et al., 2016; Heim, Mayberg, Mletzko, Nemeroff, & Pruessner, 2013) and adolescents with a history of early maltreatment (Busso et al., 2017; Gold et al., 2016; Korgaonkar et al., 2013; Lim et al., 2018). Smaller CT, but not CSA or gyrification, have also been found in anterior cingulate and orbitofrontal cortices in children (Kelly et al., 2016; Kelly et al., 2013).

Many early maltreatment studies specifically examined participants with different forms of psychopathology, such as PTSD (e.g. Thomaes et al., 2010), but differences have also been found in individuals without diagnosed disorders (Cohen et al., 2006; Dannlowski et al., 2012; Korgaonkar et al., 2013), which suggests that in these cases differences are a function of maltreatment rather than associated psychopathology. Studies that aimed to disentangle the influence of psychopathology and maltreatment have shown mixed results. Morey et al. (2015) found changes in the ventromedial prefrontal cortex to be specific for adolescents with PTSD compared to those without, suggesting that these brain changes are either PTSD-specific or mark vulnerability vs resilience following maltreatment. Sheffield et al. (2013) reported that a smaller prefrontal cortex was related to sexual abuse rather than psychosis (by comparing patients with psychotic disorder but without early maltreatment, patients with psychotic disorder and early maltreatment,
and healthy controls), which might indicate that findings of reduced prefrontal volume in psychosis potentially stem from a higher prevalence of sexual abuse in these individuals.

As for other cortical regions, the most prominent studies found changes in the primary and secondary sensory cortices, which were specific to the form of maltreatment experienced. Tomoda et al. (2011) showed that individuals exposed to parental verbal abuse, but no other forms of reported maltreatment showed increased volume of the primary auditory cortex. The researchers also found that individuals who had reported witnessing domestic violence or sexual abuse had smaller volumes of the visual cortex (Tomoda, Navalta, Polcari, Sadato, & Teicher, 2009; Tomoda, Polcari, Anderson, & Teicher, 2012). Heim et al. (2013) found that childhood sexual abuse was associated with cortical thinning in the part of the primary somatosensory cortex that represents the genital region, while emotional abuse was linked to thinning of the anterior cingulate cortex and precuneus, which have been implicated in emotion regulation (Mohanty et al., 2007). These findings, which were obtained with unbiased whole-brain approaches, provide evidence for experience-adaptive programming as described in 1.3. Instead of unspecific structural damage, brain structural associations were specific to the maltreatment experienced and might provide an adaptation in the form of sensory shielding (Heim et al., 2013).

Reductions in CT in regions other than the prefrontal cortex have also been reported in lateral parietal cortices (Lim et al., 2018) and temporal regions in adolescence following early maltreatment exposure (Busso et al., 2017; Gold et al., 2016). In children with history of early maltreatment, reduced CSA was reported for middle temporal and lingual cortices (Kelly et al., 2013) as well as the inferior parietal cortex (Kelly et al., 2016) but no local differences in CSA were found in
adolescents (Lim et al., 2018). In childhood, reduced local gyrification was observed in lingual gyrus and insula (Kelly et al., 2013) while it was increased in the right superior parietal cortex (Kelly et al., 2016).

Two meta-analyses examined the results of 12 (Lim et al., 2014) and subsequently 19 (Paquola et al., 2016) whole-brain voxel-based morphometry studies on childhood maltreatment. While Lim et al. (2014) included children, adolescent and adult cohorts, Paquola et al. (2016) specifically examined adult cohorts. Lim et al. (2014) found grey matter volume reductions in the right orbitofrontal cortex, insula, amygdala, parahippocampus and left ventrolateral prefrontal cortex in the maltreated groups. Surprisingly, larger volumes were found in the right dorsolateral prefrontal cortex and left middle occipital gyri. Paquola et al. (2016) found that adults who had experienced childhood trauma exhibited smaller volumes in the right dorsolateral prefrontal cortex. In this meta-analysis, the results were significantly moderated by age, gender, psychopathology, and type of maltreatment. The reductions in dorsolateral prefrontal cortex were mainly driven by studies of older adult cohorts with predominantly male samples indicating that the association with childhood maltreatment only manifests later in life for this brain region and is influenced by gender, with males appearing more susceptible. Moreover, no reductions in dorsolateral prefrontal cortex were found in studies defining childhood maltreatment on the basis of one traumatic event or adverse family environment and while reductions were observed in PTSD and psychosis cohorts with a history of childhood maltreatment, the results for depressed adults with a history of childhood maltreatment were mixed (Paquola et al., 2016). The influence of additional factors on the relationship between early maltreatment and brain structure might also explain the discrepancies between results of the two meta-analyses. While Lim et al. (2014) included studies in children and adults,
Paquola et al. (2016) only investigated adult cohorts. Both meta-analyses included a wide-range of definitions of early maltreatment. For adult brain structure, the larger dataset of Paquola et al. (2016) might be considered more robust, however more studies with child cohorts and longitudinal study design are needed to investigate the influence of early maltreatment on brain developmental trajectories. Nonetheless, both meta-analyses partly confirm structural changes in prefrontal cortex following childhood maltreatment as the most consistent finding but also point to potential changes in other cortical regions such as the occipital cortex and the parahippocampus.

Subcortical structures

The limbic system consists of highly interconnected cortical and subcortical structures, which are associated with emotion, memory, behaviour, motivation and olfaction (Catani, Dell'acqua, & Thiebaut de Schotten, 2013). Hippocampus and amygdala are parts of the limbic system that have long been thought to be affected by early maltreatment (Teicher & Samson, 2016). Deprivation is associated with altered emotional and social behaviour, including symptoms of DSE (Kennedy et al., 2017). These behavioural findings potentially indicate changes in the anatomy and function of the limbic system, which plays an important role in emotional processing (Grassi-Oliveira, Ashy, & Stein, 2008; Teicher & Samson, 2016). The amygdala and hippocampus develop rapidly in the first years of life (see 1.3.2.2). Both structures have a high density of glucocorticoid receptors (Morimoto et al., 1996) and are susceptible to the negative effects of stress in animal models (see 1.4.1), with hypertrophy of the amygdala and hypotrophy of the hippocampus. The hypothesis of amygdala and hippocampus as maltreatment-sensitive structures in humans is partially supported by meta-analyses of studies of participants with PTSD, which have found reduced left hippocampal volumes (Kühn & Gallinat, 2013) but no
consistent reductions in amygdalae volumes (Kühn & Gallinat, 2013; Woon & Hedges, 2009).

Smaller hippocampal volumes in individuals who experienced early maltreatment or early life stress are often regarded as one of the most consistent findings in this field of research (Humphreys et al., 2018; Lawson et al., 2017; Opel et al., 2014; Paquola et al., 2017), although negative findings do exist (Cohen et al., 2006; Korgaonkar et al., 2013). Results are more mixed for amygdala volumes (Hart & Rubia, 2012; McCrory, De Brito, & Viding, 2010; Teicher & Samson, 2016). For both structures, the complexity of findings becomes clear when comparing multiple meta-analyses. Paquola et al. (2016) included ROI studies of adult hippocampus (17 studies) and amygdala (13 studies). They found that early maltreatment was associated with reduced volumes of both structures. Another meta-analysis including ROI analyses for adult hippocampus (15 studies) and amygdala (7 studies) found smaller hippocampal volumes but no changes in amygdala volumes following early life adversity (Calem, Bromis, McGuire, Morgan, & Kempton, 2017). Yet another meta-analysis (49 studies) found that hippocampal volumes were smaller in adults with a history of childhood maltreatment but not in children (Riem, Alink, Out, Van Ijzendoorn, & Bakermans-Kranenburg, 2015). Moreover, hippocampal changes were most pronounced when maltreatment occurred during middle childhood, while early childhood and adolescence appeared to be less sensitive periods (Riem et al., 2015). A recent mega-analysis of the ENIGMA consortium, which included 958 adults with depression and 2078 healthy controls found no indication that childhood maltreatment as measured with the CTQ is associated with either smaller hippocampal or amygdala volumes (Frodl et al., 2017). It should be noted that ROI analyses might overestimate the strength of the effects (Lim et al., 2014). In the previously described whole-brain meta-analyses,
Lim et al. (2014) found reductions in a cluster of regions that included the right amygdala and parahippocampus, but not the hippocampus itself. Paquola et al. (2016) found smaller volumes in the right hippocampus but no differences in amygdalae volumes. The heterogeneity of these findings might stem from several limitations. Most importantly, a wide range of study designs were employed, which varied especially in terms of the type of maltreatment studied (Lim et al., 2014). Paquola et al. (2016) report that findings were moderated by age, gender, type and severity of maltreatment as well as psychopathology. For example, smaller hippocampal volumes are generally found in adult cohorts with PTSD and a history of childhood maltreatment but not in child cohorts (McCrory et al., 2010), consistent with the meta-analysis by Riem et al. (2015). There is evidence for attenuated hippocampal growth throughout adolescence following early maltreatment (Paquola et al., 2017), which might explain this discrepancy between child and adult cohorts.

Amygdala alterations might be age dependent. All meta-analyses except Lim et al. (2014) included adult cohorts only. Teicher and Samson (2016) hypothesised that the variability in study results might be explainable by an early increase of amygdala volumes in childhood caused by early maltreatment, which is then followed by volumetric decreases throughout development (possibly linked to recovery in adolescence and adulthood). Evidence for this hypothesis comes from studies that find larger amygdala volumes in children from low socioeconomic backgrounds (Noble, Houston, Kan, & Sowell, 2012) and larger amygdala volumes in children with chronically depressed mothers (Lupien et al., 2011). Regarding psychopathology, Paquola et al. (2016) reported reduced amygdalae volumes associated with early maltreatment in patients with psychosis as well as mood disorders, while there was no significant relationship between amygdalae volumes and early maltreatment in healthy cohorts. Moreover, the relationship between
amygdalae volumes and early maltreatment seems to be influenced by adult stress, with a positive association between amygdala volumes and combat exposure in veterans without a history of childhood trauma while the association is negative in veterans with a history of childhood trauma (Kuo, Kaloupek, & Woodward, 2012). Paquola et al (2016) conclude that a combination of childhood maltreatment and high levels of adult stress (as apparent in psychiatric cohorts) is related to reduced amygdalae volumes.

A smaller number of studies have investigated other subcortical structures, such as the basal ganglia. The caudate nucleus and putamen form the striatal part of the basal ganglia and changes in function and developmental trajectories of these structures have been linked to neurodevelopmental disorders such as ADHD (Durston et al., 2003; Hoogman et al., 2017) and ASD (Langen et al., 2009). Early maltreatment has also been associated with an increased risk of these disorders (Sonuga-Barke et al., 2017).

Multiple studies have found alterations in the caudate nucleus following early maltreatment. Smaller caudate volumes were found in healthy adolescents and adults with a history of childhood maltreatment (Cohen et al., 2006; Dannlowski et al., 2012; Edmiston et al., 2011). Moreover, the previously described mega-analysis of the ENIGMA-Depression consortium, found smaller caudate volumes to be related to early maltreatment in women only, with emotional and physical neglect showing the strongest effects (Frodl et al., 2017). It would be interesting for future studies to investigate the mediating and moderating links between early maltreatment, ADHD, ASD and basal ganglia volumes including the caudate nucleus.
1.4.3.3 White matter

The corpus callosum, the largest white matter tract, which connects the two hemispheres, has most consistently been shown to be sensitive to early maltreatment with smaller FA values observed in exposed children and adults (McCrory et al., 2010; Teicher & Samson, 2016). Other white matter tracts that show smaller FA following reported early maltreatment include the superior longitudinal fasciculus (Benedetti et al., 2014), which connects prefrontal and parietal regions and has been implicated in neurodevelopmental disorders such as ADHD (Wu et al., 2016). FA is also reduced in the uncinate fasciculus, which connects limbic regions and the orbitofrontal cortex, in individuals with a history of early maltreatment (Benedetti et al., 2014; Huang, Gundapuneedi, & Rao, 2012). Abnormalities in this tract have been associated with psychiatric disorders such as generalized anxiety disorder and depression (Bhatia, Henderson, Hsu, & Yim, 2018; Tromp et al., 2012).

1.4.3.4 Grey matter networks

Two studies investigated structural covariance of cortical thickness with graph theory measures and reported early maltreatment-related changes (Sun et al., 2018; Teicher, Anderson, Ohashi, & Polcari, 2014). Teicher et al. (2014) reported altered centrality, a measure of the degree of connectedness of a brain region, across multiple brain regions in previously maltreated adults – centrality was reduced in the anterior cingulate cortex while it was increased in right precuneus and right anterior insula. Sun et al. (2018) found that early maltreatment was related to reduced centrality in the right temporal pole and increased centrality in the left supramarginal gyrus. While the former effect was apparent in maltreated youth independent of PTSD status, the latter was specific to those without PTSD - highlighting the psychopathological specificity of such effects (Sun et al., 2018).
1.4.4 Early maltreatment and brain function

As this thesis focuses on brain structure, functional alterations associated with early maltreatment will only be reviewed briefly (for an in-depth review see McCrory et al., 2017). Functional MRI (fMRI) studies show that brain regions involved in threat or emotion processing are abnormally hyper-active in individuals with a history of early maltreatment, especially in the amygdala and other social processing regions such as the superior temporal cortex and insula (Hein & Monk, 2017). This is thought to reflect an adaptive calibration towards higher alertness to threat (McCrory et al., 2017). Moreover, there is a blunted response to reward, particularly in the striatum, which mirrors the functional response of individuals with depression. Studies investigating emotion regulation report higher activation in maltreated individuals in the anterior cingulate cortex, probably related to higher effort (McCrory et al., 2017). While brain function will never map 100% onto structural findings, it is notable that there seems to be a strong overlap between the functional and structural differences associated with early maltreatment. In conjunction, these studies provide possible psychological mechanisms for the association between structural brain alterations and changes in behaviour or clinical symptoms.

1.4.5 Methodological limitations of prior studies

This review shows that the last two decades have seen tremendous progress in the understanding of the neurobiological consequences of early maltreatment. However, many of the studies described above have multiple limitations, which limit our ability to draw causal inferences about the role of maltreatment exposures and potentially undermine the confidence in the association between maltreatment and brain structure. These limitations are related to the study designs described in 1.4.2 and will be reviewed in detail below.
1.4.5.1 Familial confounding

Familial confounding between maltreatment exposures and genetic factors within families that determine brain development and its relationship to psychopathology is a significant limitation of most of the studies described above as children mostly experienced maltreatment in their biological families (Sonuga-Barke et al., 2017). This means they are susceptible to so called passive gene-environment correlations – whereby environmental exposures and correlated brain alterations and associated psychopathology may be driven by common genetic risk factors passed from parent to child. For instance, a twin study found that monozygotic twins, where one had experienced childhood maltreatment and one had not, did not differ significantly in psychopathology (Bornovalova et al., 2013; Dinkler et al., 2017). While this might indicate substantial genetic confounding in maltreatment research, the limitations of this study should be noted: The sample size of monozygotic twins with discordant childhood maltreatment was low and maltreatment exposure was based on parent-reports, potentially leading to bias and underreporting. A review of studies investigating the interaction between candidate genes and maltreatment and psychopathology, found that maltreatment had a negative effect on psychopathology irrespective of genes (even though often attenuated by them; Maglione, Caputi, Moretti, & Scaini, 2018). However, studies included in this review were also limited by their candidate gene approach. The limitation of potential familial confounding severely restricts our ability to draw causal inferences about the effects of early maltreatment. Studies that investigate the effects of maltreatment in adoptive settings are needed to disentangle the effects of environment and genes/familial risk factors.
1.4.5.2 Retrospective reporting of maltreatment

Many of the studies above are based on retrospective reports of maltreatment, for example by using a questionnaire like the CTQ or interviews at the time of the study (many years after the maltreatment occurred). Studies that compared prospective and retrospective accounts of childhood maltreatment longitudinally, found that there was only a moderate overlap between the two (Newbury et al., 2018; Reuben et al., 2016). Only 28% of participants who had prospectively reported childhood maltreatment, continued to do so at age 18 and most individuals who retrospectively reported maltreatment had not done so during childhood (Newbury et al., 2018). Strikingly, retrospective reports were more strongly related to psychopathology, indicating two possible mechanisms: healthy adults might tend to “forget” or underplay childhood maltreatment while adults with psychopathology might tend to overreport retrospectively (Hardt & Rutter, 2004; Newbury et al., 2018; Reuben et al., 2016). This poses a potential caveat for the high association seen between psychopathology and early maltreatment in these studies. Studies in this review which assessed early maltreatment exposure prospectively and/or used official records by social services to corroborate self-report measures (e.g. De Bellis et al., 2015; De Bellis et al., 2002; Kelly et al., 2016; Kelly et al., 2013; Lim et al., 2018; Morey et al., 2015) offer a better opportunity to establish the pathway between early maltreatment, changes in brain structure and later psychopathology.

1.4.5.3 Psychopathology

Researchers have long acknowledged the confounding and overlapping effects of maltreatment and psychopathology on brain structure, but it remains extremely difficult if not impossible to separate these variables given the strong association between them. Relatedly, studies that examine whether brain structural alterations
following early maltreatment might constitute resilience and compensation or vulnerability for later disorder are scarce.

1.4.5.4 Cross-sectional design

Most of the studies reported in this review have utilised retrospective approaches and cross-sectional brain imaging, which limits the ability to infer causal inferences of early maltreatment on brain structure and functioning (Hart & Rubia, 2012). Only by using more prospective longitudinal approaches, will we be able to disentangle the effects of early maltreatment and subsequent psychopathology and to map the altered developmental trajectories of different brain regions.

1.4.5.5 Ongoing adversity

Most of the studies described above described maltreatment within a familial setting. Children who experience maltreatment within the family are likely to continue to experience maltreatment throughout development (Dunn et al., 2011). It is therefore difficult to distinguish the effects of maltreatment during putative sensitive periods of development from the effects of subsequent adverse environments.

1.4.5.6 Abuse versus neglect

It is plausible that abuse and neglect might have similar effects on brain development sharing a biological pathway of early stress exposure. However, there might be additional disparate pathways and effects on neurodevelopment that are distinct for abuse and neglect experiences (McLaughlin, Sheridan, & Lambert, 2014). In familial settings different types of maltreatment co-occur and 95% of individuals who reported parental neglect also reported at least one other form of childhood adversity (Green, McLaughlin, Berglund, Gruber, et al., 2010). This might be one of the reasons why most studies reported here did either not differentiate
between abuse and neglect and studied maltreatment in general or focussed on early abuse. Only very few studies have investigated the neurodevelopmental effects of early neglect (e.g. Wang et al., 2014). Consistent with the view that neglect constitutes a form early deprivation (McLaughlin, Sheridan, & Lambert, 2014), studies investigating individuals who have been exposed to institutionalisation in early childhood might provide some indication of the biological pathways sensitive to neglect and profound deprivation.

1.4.6 Interim conclusion

Current literature suggests that early maltreatment is associated with profound and long-term alterations in brain structure and functioning. Overall, total brain volumes tend to be smaller, with the largest regional effects seen in the prefrontal cortex and hippocampus, while results regarding the amygdala are mixed. There is also evidence for smaller caudate volumes and changes in other cortical areas such as the precuneus and parahippocampus. FA values are lower in the corpus callosum, and possibly the uncinate and superior longitudinal fasciculus, which connect many of the structures mentioned above. Abnormalities in function have also been observed, mostly in brain regions which have also shown structural abnormalities. However, interpreting these associations is complicated by the significant design limitations described above.
1.5 **BRAIN IMAGING STUDIES OF CHILDREN PLACED IN AND THEN REMOVED FROM INSTITUTIONAL DEPRIVATION**

Only a small number of maltreatment studies have used structural MRI methods to specifically examine the effects of early deprivation as a result of institutionalisation. Typically, these studies compare children, who spent a certain amount of time in institutional care, with never-institutionalised children. Therefore, these studies are able to link outcomes to a specific and time-limited period in the child’s development, during which they experienced severe deprivation (Mehta et al., 2009; Sheridan, Fox, Zeanah, McLaughlin, & Nelson, 2012; Tottenham et al., 2010).

Adoption studies can overcome many of the limitations mentioned in 1.4.5. Firstly, there is reduced risk of familial confounding, as children, caretakers in the institutions and adoptive parents are not biologically related (Sonuga-Barke et al., 2017). These study designs strengthen causal inferences, especially if the institutionalisation and later adoption was determined by external factors like it was the case with children institutionalised in Romania during the Ceaușescu regime. Under such circumstance adverse effects following institutionalisation are likely to have been *caused* by institutional deprivation rather than pre-existing genetic or prenatal risk.

Secondly, time spent in institutions can be objectively assessed based on institutional and adoption records and therefore the assessment of maltreatment exposure does not rely on subjective retrospective accounts. Deprivation experienced in these institutions can be assessed by measuring the care provided and by making inferences about factors such as subnutrition based on objective measures such as body weight.
Thirdly, precisely timed deprivation exposure allows to differentiate the effects of early maltreatment and ongoing adversity. Adoptive parents mostly provide loving and nurturing environments that stand in stark contrast to the harsh and depriving conditions of the institutions the children were adopted from (Rutter, 1998). These studies therefore allow to investigate whether early adversity is associated with long-term neurobiological changes in development despite the supportive environment post adoption strengthening inferences on the importance of sensitive periods of development.

Studies investigating institutional deprivation also have potential limitations. Most importantly, potential cofounding factors need to be investigated and controlled for. For example, it should be determined why children were placed in the institutions. It can often not be ruled out that certain traits of the infant might increase risk of institutionalisation and this could be a confounding factor when determining the effects of deprivation. In such cases it might be informative to investigate if extended deprivation duration is associated with more negative effects to strengthen causal inferences. Again, potential additional factors should be investigated, for example, time of adoption might also be associated with certain traits of the child confounding the effects of deprivation duration.

1.5.1 Early institutional deprivation and brain structure

Four relevant studies were identified which studied the effects of deprivation on brain structure in individuals who had been institutionalised and then adopted.

The Bucharest Early Intervention Project (BEIP) follows a longitudinal design and examined the neural changes associated with early deprivation (Sheridan et al., 2012; Zeanah et al., 2003). In a randomised controlled trial of foster care treatment, 136 institutionalised children were either assigned to a continued stay in a
Romanian institution or a placement into a foster family. A community control group comprised an additional 72 Romanian children at the same age who had never been institutionalised (Zeanah et al., 2003). When these children were in middle childhood (aged between 8 and 11), an MRI study was conducted to examine the effects of institutionalisation on brain structure which included 29 institutionally-reared children, 25 children in foster care and 20 children of the community control sample (Mclaughlin et al., 2014; Sheridan et al., 2012). Out of the studies identified here, the BEIP was the only prospective longitudinal study. Even though brain imaging results were cross-sectional, longitudinal measures of the biopsychosocial development were reported and measures of the conditions in the institutions and during foster care were available. Another strength of the BEIP lies in its randomised controlled design, which allows the continuous effects of placement into foster care to be investigated. However, the timing of placement into institutions was not randomised and therefore effects of duration of institutionalisation vs timing of the institutionalisation exposure cannot be differentiated.

Tottenham et al. (2010) investigated brain structure of 34 children, who had been adopted from international institutions into American families (mean age at adoption: 18.8 months) and a group of 28 never-institutionalised children. A strength of this study was that it investigated the effect of deprivation duration as a continuous as well as categorical variable (groups split at median age of adoption which was 15 months). Moreover, the correlation between amygdala volumes and anxiety symptoms was tested in a subsample of this study even though with small sample sizes which did not allow to compare institutionalised and comparison groups.

In another study, Hodel et al. (2015) examined the effects of early deprivation on brain structure in a sample of 110 post-institutionalised children (median age at
adoption was 12 months) in comparison to 62 non-adopted children, all aged between 12 and 14 years. A subset of this sample was further investigated in Herzberg et al. (2018). A strength of this study was that it investigated both cortical thickness and surface area, however only for brain areas that had shown group differences in terms of volume. Regions that showed differences in only thickness or surface area but not volume would not have been identified. Another caveat of this study was that it excluded children with diagnosed neurodevelopmental disorders from the comparison group. Investigations of the relationships between deprivation, brain structure and psychopathology were therefore limited. As a strength, this study investigated the effect of deprivation duration albeit only as a categorical comparison between adoption before or after 15 months.

In another study, 36 previously institutionalised internationally adopted children (mean age at adoption: 30 months) were compared to 41 non-adopted children (Hanson et al., 2015). Total white matter volumes for a subset of this sample were reported in Hanson et al. (2013). A strength of this study was that amygdala and hippocampal volumes were segmented manually which is considered the gold standard of segmentation of these volumes. On the other hand, only amygdala and hippocampal volumes were reported in this study. These volumes were correlated with behavioural problems but no other neurodevelopmental or psychiatric outcomes.

All four studies overcome many of the limitations of the observational maltreatment studies described above as the institutionalisation and adoption strengthen inferences on early maltreatment effects with reduced risk of familial genetic confounding. However, these studies have limitations, too. First, except for the BEIP, all studies described here were based on internationally adopted children. The reasons that determined the placement in the institutions and subsequent
adoption are unclear in these studies. It cannot be ruled out that placement in the institutions might be associated with pre-existing disorder risk because of genetic or environmental influences. Likewise, adoption and associated deprivation duration might to some extent be associated with traits of the child rather than purely external circumstances. None of the studies described here have a comparison group of individuals who were adopted but did not experience institutional deprivation. This makes it difficult to distinguish the experience of adoption per se from institutional deprivation. Out of the studies described here only the BEIP performed comprehensive whole-brain approach analyses while the other studies focused on regions of interest, mainly total brain volume and amygdala and hippocampal volumes. Consequently, it is difficult to draw inferences on differences in regions outside or not aligned with these regions of interest. An overview of the studies investigating brain structure and institutional deprivation can be found in Table 1.1.
Table 1.1: Studies investigating the effects of institutional deprivation on brain structure.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Age when tested (in years)</th>
<th>median age at adoption (in months)</th>
<th>Strengths</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(McLaughlin, Sheridan, Winter, et al., 2014; Sheridan, Fox, Zeanah, McLaughlin, &amp; Nelson, 2012)</td>
<td>29 institutionally-reared children, 25 in foster care, 20 comparison</td>
<td>8 – 10</td>
<td>NA</td>
<td>prospective longitudinal design; randomised foster care; whole brain analysis</td>
<td>Cross-sectional imaging, no investigation of cortical surface area or gyrification</td>
</tr>
<tr>
<td>(Tottenham et al., 2010)</td>
<td>34 PI, 28 comparison</td>
<td>5 – 15</td>
<td>15</td>
<td>(Categorical) deprivation duration</td>
<td>Regions of interest only; internationally adopted</td>
</tr>
<tr>
<td>(Herzberg et al., 2018; Hodel et al., 2015)</td>
<td>110 PI; 62 comparison</td>
<td>12 – 14</td>
<td>12</td>
<td>(Categorical) deprivation duration</td>
<td>Regions of interest only; internationally adopted</td>
</tr>
<tr>
<td>(Hanson et al., 2013; Hanson et al., 2015)</td>
<td>36 PI; 41 comparison</td>
<td>9-14</td>
<td>35</td>
<td>Manual tracing of subcortical volumes</td>
<td>Regions of interest only; internationally adopted</td>
</tr>
</tbody>
</table>

PI – post-institutionalised sample; NA – Not available

1.5.1.1 Total brain volume

In the BEIP, participants who had spent any time in a Romanian institution showed overall reductions of 6.5% in grey matter volumes (Mclaughlin et al., 2014; Sheridan et al., 2012). White matter volumes were 6.4% smaller, but only for those children who experienced extended institutionalisation rather than being placed into a foster family, suggesting a potential for recovery in white matter following reinstatement of parental care. Hanson et al. (2013) reported smaller overall white matter volumes for post-institutionalised children (while grey matter volumes were not investigated). However, in the study of Tottenham et al. (2010), no differences in overall cortical volumes were found between post-institutionalised and non-adopted children. None
of the studies above examined deprivation duration as a continuous predictor or tested whether changes in global brain measures predicted behavioural outcomes.

1.5.1.2 Grey matter

Cortex

Widespread reductions in CT were found in prefrontal, parietal and temporal regions for children who had ever spent time in institutional care compared to the community control group in the BEIP (McLaughlin, Sheridan, Winter, et al., 2014). Moreover, smaller CT in the lateral orbitofrontal cortex, inferior parietal cortex, precuneus, superior temporal cortex and lingual gyrus were found to mediate the relationship between institutionalisation and elevated ADHD symptoms (McLaughlin et al., 2014). The effects of deprivation duration were investigated continuously, however there were no significant associations with cortical thickness after controlling for multiple comparisons. Hodel et al. (2015) reported smaller cortical volumes in all lobes in the adoptees group, when controlling for total intracranial volume. These effects were strongest in the prefrontal cortex and predominantly based on changes in cortical surface area rather than thickness. There were no differences between early and late adopted groups. The other studies did not investigate specific cortical regions.

Subcortical structures

Tottenham et al. (2010) reported larger amygdala volumes in post-institutionalised children, who had spent an extended period (more than 15 months) living in institutions. On the other hand, Hanson et al. (2015) and Hodel et al. (2015) found smaller left amygdala volumes (but no differences in right volumes) in the post-institutionalised group. Only two studies correlated subcortical volume differences with behavioural outcomes, with larger deprivation-related amygdala volumes associated with higher anxiety (Tottenham et al., 2010) and smaller deprivation-
related amygdala volumes linked to more behavioural problems (Hanson et al., 2015). While two studies found no significant differences for hippocampal volumes (Hanson et al., 2015; Tottenham et al., 2010), Hodel et al. (2015) reported smaller bilateral hippocampi in the late adopted children with more than 12 months of institutional care. In contrast, no differences in amygdala, hippocampal or basal ganglia volumes were found in the ever-institutionalised sample of the BEIP compared to the never-institutionalised community control sample (Sheridan et al., 2012). All studies adjusted for either total intracranial, brain, or grey matter volume.

These results mirror the mixed findings from non-adoption/foster care maltreatment studies. All studies described here considered children and adolescents but not adults. Most found no differences in hippocampal volumes, which is in line with non-adoption maltreatment studies of child and adolescent cohorts. While the study by Tottenham et al. (2010) might confirm the hypothesis of initially enlarged amygdala volumes in childhood (Teicher & Samson, 2016), others do not find an effect or indeed smaller volumes. Amygdala volumes will therefore most likely show a more complex relation to early deprivation, possibly moderated by psychopathology, genetic vulnerability and subsequent environmental stress.

1.5.1.3 White matter

The BEIP cohort underwent DTI scanning when around 8 years old (Bick et al., 2015). There was some indication for abnormalities in FA and additional DTI measures of multiple tracts including the corpus callosum, cingulum, fornix, anterior and superior corona radiata and external capsule. With the exception of the corpus callosum and superior corona radiata, differences in these tracts were only present when comparing the group of children in continued institutional care to the never-institutionalised group, suggesting potential for recovery for the foster care group (Bick et al., 2015). Hanson et al. (2013) reported lower FA values in multiple white
matter tracts connecting the temporal lobe and the prefrontal cortex. Two additional DTI studies will be considered: Govindan, Behen, Helder, Makki, and Chugani (2010) found smaller FA in the uncinate and superior longitudinal fasciculi in 17 previously institutionalised children compared to 15 age-matched healthy non-adopted controls. Reduced FA in the uncinate fasciculi was also reported in the DTI study by Kumar et al. (2014), who compared 36 previously institutionalised children (mean age at adoption: 26 months) to 16 non-adopted children.

1.5.1.4 Grey matter networks

No studies were identified that investigated the impact of early institutional deprivation on structural covariance networks.

1.5.2 Early institutional deprivation and brain function

Functional abnormalities in similar brain regions as reviewed earlier (1.4.4; McCrory et al., 2017) – namely the amygdala and hippocampus – have been found following institutional deprivation. Amygdala activity was found to be altered with higher activity in response to fearful faces in previously institutionalised children compared to non-adopted controls (Tottenham et al., 2011). Moreover, an additional fMRI study which investigated amygdala response to the child’s mother’s face compared to a stranger’s face found that amygdala response to the mother’s face was typically larger in a comparison group, but no difference in amygdala response was seen in formerly institutionalised children (Olsavsky et al., 2013). Finally, Silvers et al. (2016) found that previously institutionalised children and adolescents showed increased functional connectivity between the hippocampus and prefrontal regions during in an aversive-learning fMRI task compared to age-matched non-adopted controls. This coupling predicted reduced anxiety symptoms over a 2-year period, suggesting it might be adaptive.
1.5.3 Interim conclusion

The profound impact of early institutional deprivation manifests in substantially smaller total grey and white matter volumes. There is some indication that prefrontal areas are most strongly affected, which is in agreement with previously described studies of early maltreatment. Even though the amygdala and hippocampus are the key subcortical areas hypothesized to be sensitive to early maltreatment, institutional deprivation studies mostly find no differences in hippocampal volumes while there is some evidence for initially increased amygdala volumes. The absence of effects on hippocampal volumes could be age-dependent as only children cohorts were investigated and early maltreatment studies which found effects on hippocampal volumes investigated adults (Teicher & Samson, 2016). Initial findings also suggest that the effects of early deprivation on brain structure might mediate behavioural symptoms. According to these findings, deprivation-related alterations in cortical thickness in frontal, parietal and temporal regions and amygdala volume might indicate impairment as they were associated with more symptoms and behavioural problems. The very few DTI and fMRI studies on institutional deprivation partly confirm the findings of non-adoption maltreatment studies, showing smaller FA in uncinate and superior longitudinal fasciculus but no difference in the corpus callosum and functional differences in the limbic system. However, no studies have yet investigated the effects of institutional deprivation on adult brain structure. Moreover, the role of deprivation duration on brain structure remains poorly understood and no studies have systematically investigated whether brain-behaviour correlations represent manifest disorder risk, latent cognitive vulnerability or compensation.
1.6 **English and Romanian Adoptees (ERA) Study**

Prospective longitudinal studies of individuals who were exposed to deprivation within institutions during early childhood and then adopted into supportive and nurturing environments allow the neurodevelopmental effects of early adverse environments to be studied in a design that is largely independent of other factors such as familial genetic risk and ongoing adversity.

The English and Romanian Adoptees (ERA) study is an example of such a study design and has provided unique insights into the effects of early deprivation on human development (Rutter et al., 2010). The study follows Romanian adoptees, who were exposed to severely depriving conditions including malnutrition, minimal social contact and limited cognitive stimulation during their time in the institutions of the Ceaușescu regime (Sonuga-Barke et al., 2008). After the fall of the regime many of these children were adopted into families originating from several different countries, including the UK, thus bringing about a drastic and sudden improvement of the children’s environment. Capitalising on this unique “natural experiment”, the ERA study is a comprehensive, longitudinal study examining the development of some of these children who were adopted into UK families (Rutter, Kumsta, Schlotz, & Sonuga-Barke, 2012). The ERA study aims to develop a better understanding of the effects of early adversity on psychological, physiological as well as social and emotional development.

The original sample comprised 165 Romanian and – as a control group – 52 non-deprived UK adoptees. The age at UK entry – and therefore at adoption – ranged from 0 to 42 months for the Romanian adoptees (mean age: 6.6 months). All UK adoptees were adopted before 6 months of age and were not exposed to institutional care or other substantial deprivation experiences (mean age: 2.5 months; Rutter et al., 2010). Inclusion of this comparison group allows to isolate the
effects of deprivation from adoption per se (Rutter, 1998). The adoptees have been studied at 4, 6, 11 and 15 years of age (Rutter et al., 2010) and recently as young adults (aged between 22 and 25 years) in the latest follow-up-study (Kennedy et al., 2016; Sonuga-Barke et al., 2017). The current follow-up phase has also been extended to include a neuroimaging study: the ERA Brain Imaging Study (ERABIS).

The aim of ERABIS is to gain comprehensive insights into the long-term effects of early institutional deprivation on brain structure and functioning in young adulthood. Compared to studies described in 1.5 that investigate internationally adopted children, causal inferences in ERA are strengthened because placement into institutions and age at adoption were determined by external factors (e.g. the conditions under and the fall of the regime). Most children entered the institutions within the first weeks of life making it unlikely that they were placed because of pre-existing conditions (Rutter et al., 2012). Likewise, Romanian authorities decided which children were considered for adoption rather than adoptive parents and the age at which children were adopted was largely determined by their age at the time of the fall of the regime (Rutter, 1998). These circumstances reduce the risk that adoption and age at adoption were influenced by temperamental characteristics of the child or other internal factors. It should also be noted that, as a consequence of being placed into institutions shortly after birth, deprivation duration was a function of the child’s age at adoption. This means that we cannot examine whether the effects of deprivation duration differ depending on their co-occurrence with sensitive periods of early brain development other than highlighting the importance of the first years of life. Table 1.2 summarises main characteristics of ERA compared to the BEIP, the only other prospective longitudinal study that investigates the impact of early institutional deprivation on brain development.
Table 1.2: Characteristics of the English and Romanian Adoptees Study and the Bucharest Early Intervention Project.

<table>
<thead>
<tr>
<th>English and Romanian Adoptees Study</th>
<th>Bucharest Early Intervention Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institutionalisation during Ceaușescu regime</td>
<td>Institutionalisation after the fall of the Ceaușescu regime</td>
</tr>
<tr>
<td>▪ Extreme institutional deprivation but limited information on specific conditions</td>
<td>▪ Relatively less extreme deprivation but more detailed assessment of conditions within the institutions</td>
</tr>
<tr>
<td>Group of Romanian children adopted into UK families and within UK adopted control group</td>
<td>Within Romania, random assignment to continued stay in institutions or foster care and community control group</td>
</tr>
<tr>
<td>▪ This allowed to investigate duration of deprivation within the whole group of Romanian adoptees.</td>
<td>▪ The continued institutionalisation group allowed to investigate the effects of ongoing deprivation.</td>
</tr>
<tr>
<td>Cross-sectional brain imaging in young adulthood</td>
<td>Cross-sectional brain imaging in childhood</td>
</tr>
<tr>
<td>▪ This allowed to investigate effects that persisted into adulthood – more than 20 years after institutionalisation has ended, but not whether earlier effects were present that disappeared</td>
<td>▪ This allowed to investigate effects relatively close after exposure and might therefore capture effects during childhood but not whether these persist</td>
</tr>
</tbody>
</table>
1.7 Previous findings of ERA

Previous results from the ERA study up to mid-adolescence showed a rapid catch-up and substantial recovery in many of the Romanian adoptees (Rutter, 1998). At each point of assessment recovery was partly dependent on the amount of time they had spent living in institutions. Specifically, there was a step-wise increase in severity of impairment amongst children who had been institutionalised for more than 6 – 12 months. Children who spent less than 6 months in the institutions were mostly indistinguishable from UK adoptees in psychological and physical development, and this was often the case as early as at age 6 years (Rutter & O'Connor, 2004). However, a substantial proportion of the Romanian adoptees who had spent more than 6 months in institutional care and therefore experienced extended deprivation, showed persisting and distinguished patterns of problems. These problems, which were previously defined as deprivation specific pattern (Kreppner et al., 2010), comprised increased symptoms of ADHD, ASD, DSE and cognitive impairment (Kreppner et al., 2010; Kumsta, Kreppner, et al., 2010).

In the latest young adult follow-up study, the adoptees, who were now aged between 22 and 25 years, were assessed again (Sonuga-Barke et al., 2017). The initial results show a strong persistence of many of these problems into young adulthood. Strikingly, Romanian adoptees who had spent more than 6 months in institutional care showed a seven times higher risk for meeting DSM-5 criteria for ADHD in adulthood compared to adoptees who had only experienced deprivation for a limited time (less than 6 months in Romanian institutions) and UK adoptees who had never experienced institutional deprivation (Kennedy et al., 2016). Moreover, adoptees with extended deprivation showed persistently higher rates of ASD and DSE symptoms, as well as an emergence of elevated rates of emotional symptoms throughout adolescence and young adulthood (Kennedy et al., 2017;
Sonuga-Barke et al., 2017). In contrast, cognitive impairment remitted across development with no higher rates of individuals with IQ below 80 in the group of Romanian adoptees with extended deprivation compared to UK adoptees in young adulthood.

These results demonstrate the adverse long-term effects of early deprivation on mental health and well-being despite the nurturing and supportive environment provided by the vast majority of the adoptive families (Sonuga-Barke et al., 2017). Ongoing research by the ERA team is examining which neurobiological factors are responsible for these persisting effects. For example, severe deprivation in our sample has also been linked to changes in the epigenome (Kumsta et al., 2016) and the cortisol awakening response (Kumsta et al., 2017), which might help us to understand the mechanisms underlying the behavioural, as well as the neural, patterns observed.

Considering the persisting and multi-faceted behavioural changes that occur following early maltreatment (Kreppner et al., 2010), it is reasonable to hypothesise that these are also associated with global or widespread changes in brain structure. In those raised in institutional settings, the lack of cognitive stimulation as well as emotional neglect may shape the child’s brain development. Moreover, many children in ERA suffered from subnutrition as indicated by severely delayed overall physical growth (Sonuga-Barke et al., 2008), which likely also affected brain growth. Rutter et al. (2012); and Sonuga-Barke et al. (2008) examined head circumference, which can be considered an index of brain growth (Bartholomeusz, Courchesne, & Karns, 2002), in the ERA sample. Compared to UK adoptees, the Romanian adoptees showed significantly smaller head circumferences at the age of adoption as well as at the age of 6 years. At the age of 15, despite substantial catch-up, the group of adoptees who had experienced subnutrition during institutional care
showed reduced head circumference, irrespective of length of deprivation. However, head circumference was reduced even in the absence of subnutrition in the group of adoptees who had experienced extended deprivation (Rutter et al., 2012). As indicated by these findings on head circumference, it can be hypothesized that psychosocial deprivation as well as subnutrition experienced during institutional care lead to substantial and long-lasting reductions in global brain volume.

### 1.7.1 ERA Brain Imaging Pilot Study

ERA has performed a brain imaging pilot study when adoptees were around 16 years old to gain initial insights on the structural as well as functional brain differences following institutional deprivation. In this study, 14 participants of the adoptees group with extended deprivation (>6 months) were compared to 11 age-matched non-adopted controls without psychopathology (Mehta et al., 2009).

The pilot study found that institutional deprivation was associated with reductions of 15% and 18% in total grey and white matter volumes respectively (Mehta et al., 2009). Moreover, amygdala volumes were significantly larger in the adoptees than the controls, especially on the right side (Mehta et al., 2009). However, and against expectations, left amygdala volume was negatively correlated with the time spent in institutional care. No group differences were found for hippocampal volumes. For white matter tracts, no differences in corpus callosum size following institutional deprivation (Mehta et al., 2009). Functional brain imaging revealed reduced activation in the striatum during reward anticipation in the post-institutionalised group (Mehta et al., 2010). Together these findings provide initial evidence for the strong neurobiological programming effects of institutionalisation on brain structure and functioning.
1.8 AIMS

1.8.1 Aims of ERA Brain Imaging Study (ERABIS)

ERABIS is the first study to investigate the effects of early institutional deprivation on brain development in a cohort of young adults. Participants in the ERA study spent the first months or years of their lives in Romanian institutions under extremely depriving conditions before being adopted into mostly stable, caring and supportive families (Sonuga-Barke et al., 2017). In contrast to many other studies, where participants experienced maltreatment over an extended period of their childhood, this unique natural experiment allows us to link brain alterations to a distinct time interval in the children’s development during which severe deprivation was experienced thus separating out the effects of early from on-going adversity. ERABIS aims to assess structural and functional alterations in putative deprivation-sensitive networks. Moreover, we aim to model the relationship between the duration of institutional deprivation and predicted brain alterations. Ultimately, ERABIS aims to gain comprehensive insights into fundamental questions about neuroplasticity, sensitive periods, the heterogeneity and specificity of deprivation effects and their relation to adult psychopathology.

1.8.2 Aims of this thesis

For my PhD project which has been carried out within the broader ERABIS project, I will attempt to complement these aims by specifically investigating the effects of early deprivation on morphometric measures of cortical regions (thickness, surface area and volume) and subcortical regions (volume, Chapters 3-5) as well as on structural covariance, as defined by inter-regional correlations between these measures (Chapter 6). In doing so, I aim to gain a more detailed understanding of
the long-term effects of institutionalisation per se as well as deprivation duration on adult brain structure and structural covariance. Moreover, I aim to investigate the relationship between the predicted brain morphometric changes and the behavioural and psychopathological symptoms observed in the ERA sample, to examine whether brain structural alterations following deprivation represent potential compensatory effects, manifest disorder risk or latent cognitive vulnerability to disorder (Chapter 4). This analysis will be extended to examine whether deprivation-related neurodevelopmental symptoms have distinct brain structural correlates compared to idiopathic (non-deprivation-related) symptoms (Chapter 5).
CHAPTER 2

METHODS
2.1 **Highlights**

- There was no strong indication for selective attrition in ERABIS, however, in the extended deprivation group, individuals who were included in ERABIS had lower ASD symptoms at age 6 compared to those who dropped out.
- Structural T1 scans of 67 Romanian adoptees and 21 UK adoptees were included in the analyses reported in this thesis.
- Cortical thickness, surface area, volume, local gyrification indices and subcortical volumes were extracted with FreeSurfer 6.0.
- Compared to manual segmentation of a subset of structural scans, FreeSurfer 6.0 provided satisfactory accuracy for hippocampal and amygdala segmentations and was therefore applied to the whole sample.
2.2 Overview

All empirical chapters share a substantial part of their methodological approach and the common elements will be reviewed in here in an introductory methods chapter. First, this chapter will introduce the recruitment strategy and description of the sample. Second, it will provide an overview of the measures used and the study protocol. Third, the magnetic resonance imaging (MRI) data acquisition and processing procedures will be described. Finally, I will report two sets of analyses, which assessed generalisability and accuracy of our findings. The first is a selective attrition analysis, the second is a comparison of manual versus automated segmentation to assess whether the latter (which is more efficient and potentially less subject to experimenter bias) is sufficiently accurate to be used in the whole sample.
2.3 **PARTICIPANTS**

The original ERA sample included 165 Romanian adoptees (Rom) and a non-deprived comparison group of 52 UK adoptees (UK) who had been placed for adoption before 6 months (Rutter, 1998). Out of these, 81 Rom and 23 UK took part in ERABIS. 11 Rom, who had never been institutionalised but were directly adopted from Romanian families, were excluded from the analyses reported in this thesis: Their brain volumes showed a significantly higher variance compared to the previously institutionalised Rom indicating that their pre-adoptive environment might not be comparable. Moreover, 2 UK and 3 Rom were excluded from the analyses due to missing structural MRI data (due to participants only being willing to take part in the neuropsychological assessment or not being eligible for scanning or technical failure). The final sample comprised 67 Rom (40.6% of the original sample, 50.7% female, mean age=25.3 years, SD age=1.1 years) and 21 UK (40.4% of the original sample, 38.1% female, mean age=24.4 years, SD age=1.0 years). Most Rom entered the institutions in the first few weeks of life. Deprivation duration was therefore estimated based on the age (in months) at which adoptees first entered a household in the UK. For the Rom group seen in ERABIS, deprivation duration ranged between 3 and 41 months.

Data collection took place at the Centre for Neuroimaging Sciences at King’s College, London. All participants gave written informed consent to participate and received a £100 Amazon voucher as reimbursement for their time. ERABIS received ethical approval from the ethics committee of the University of Southampton and the Camberwell - St. Giles NHS Research Ethics Committee (Ethics No.: 14/LO/0477).
2.4 Measures

ERA is a prospective longitudinal study which draws on comprehensive recordings of physiological measures, neuropsychological assessments and extensive interviews spanning more than 20 years. Here, I only introduce the measures subsequently used for the analyses reported in this thesis. A visual summary can be seen in Figure 2.1.

![Diagram showing measures across different study waves](image)

**Figure 2.1: Overview of the measures used in this thesis.** Colours of measures indicate the study wave at which they were assessed: orange – UK entry, yellow – age 6, blue – ERA young adult follow-up, green – ERABIS.

2.4.1 Physiological measures

2.4.1.1 Measures obtained at UK entry

*Birth weight*

Birth weight (in kg) was obtained from Romanian reports (The English & Romanian Study Team, 2010).
Subnutrition

Weight was recorded when children entered the UK shortly after leaving their institutions (Sonuga-Barke et al., 2008). At that time approximately 69% of the Romanian adoptees suffered from subnutrition with weight at more than 1.5 SDs below UK norms. Subnutrition was either used as a continuous variable (in SD relative to UK norms) or as a categorical predictor (subnutrition present if below 1.5 SDs below UK norms).

2.4.1.2 Young adult follow-up

Head circumference and height

Head circumference and height (in cm) were recorded.

Polygenic scores for intracranial volume

To study whether differences in total brain volume could be explained by pre-existing genetic risk, we calculated polygenic scores for intracranial volume. DNA samples were obtained with self-collection buccal cell kits and genotyped with Illumina Psych Arrays. Polygenic scores for intracranial volume were calculated with PRSice (Euesden, Lewis, & O’Reilly, 2015) and based on summary statistics from the ENIGMA genome-wide association study (Adams et al., 2016). Individual scores represent sum scores of the effect sizes of the single nucleotide polymorphisms (SNP). The optimal (explaining most of the phenotypic variance) probability threshold for inclusion of SNPs was based on the total brain volume data available in this sample.

2.4.2 Deprivation-specific neurodevelopmental problems

Throughout previous ERA follow-ups, symptoms of attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD) and disinhibited social engagement (DSE) were significantly associated with deprivation (Sonuga-Barke et
al., 2017). Cognitive impairment (as indicated by IQ) was associated with deprivation earlier in development but had remitted considerably by young adulthood (Sonuga-Barke et al., 2017).

2.4.2.1 Follow-up at age 6

For the selective attrition analyses (see 2.8), I used the following measures on deprivation-specific neurodevelopmental problems at age 6.

**ADHD symptoms**
ADHD symptoms were measured with the parent-rated Revised Rutter scale (Elander & Rutter, 1996). The items assessed whether children displayed symptoms of inattention or overactivity.

**ASD symptoms**
ASD symptoms were assessed with 15 items of the parent-rated Social Communication Questionnaire (SCQ), which were selected because they were considered developmentally appropriate at all ages of the ERA follow-ups (0 - 15 scale; Rutter, Bailey, & Lord, 2003; Sonuga-Barke et al., 2017).

**DSE symptoms**
DSE symptoms were rated based on parents' responses to three interview questions which explored whether the child showed a “lack of differentiation among adults with respect to the child’s social response to them”, “clear indication that the child would readily go off with a stranger” and “lack of checking back with the parent in anxiety-provoking situations”. Responses to each question were rated as endorsed (1) or not endorsed (0; 0-3 scale; Sonuga-Barke et al., 2017).

**IQ**
Intelligence quotient (IQ) was assessed as a measure of cognitive impairment with the McCarthy Scales of Children's Abilities General Cognitive Index (McCarthy, 1970) which is a widely-used test of general ability for children aged 2 to 8.
2.4.2.2 Young adult follow-up

The following measures were collected during the latest ERA follow up study, the ERA young adult follow-up, when participants were aged between 22 and 26 years. For a list of all items used per measure see Appendix A.

**ADHD symptoms**

ADHD symptoms were measured with 20 parent-rated items of the Conners Comprehensive Behaviour Rating Scales (0-18 scale; Conners CBRS; Conners, Pitkanen, & Rzepa, 2011; Kennedy et al., 2016). Symptoms reflect the 18 DSM-5 ADHD symptoms and were adapted for young adults with permission from the copyright holders (Kennedy et al., 2016).

**ASD symptoms**

ASD symptoms were assessed with the same 15 items of the parent-rated SCQ as at age 6.

**DSE symptoms**

DSE symptoms were rated based on parents’ responses tapping into the concept of being “too friendly towards strangers”, showing “inappropriate intrusiveness” and being “unaware of social boundaries”. Responses were rated as endorsed (1) or not endorsed (0; 0-3 scale; (Sonuga-Barke et al., 2017).

2.4.2.3 ERABIS

**IQ**

Intelligence quotient (IQ) was assessed with the full version of the Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-II; Wechsler, 2011) which is a widely-used and reliable test of general intelligence.
2.4.3 Neuropsychological assessment in ERABIS

The ERABIS protocol comprised an extensive battery of neuropsychological tests. This thesis includes results of the Memory for Intentions Test (assessing prospective memory), a modified Go-NoGo task (indexing proactive inhibition) and the empathic accuracy task. For a list of participant instructions for each task see Appendix B.

**Prospective memory**

Prospective memory was assessed with the Memory for Intentions Test (MIST; Woods et al., 2008). For this test, participants were instructed to solve a cross-word puzzle. They were also asked to follow the experimenter’s instructions throughout the test and to use a clock that was placed on the table as a guide on when to do each of the tasks. Throughout the test, participants were required to remember to complete tasks either at specified times (short interval of 2 min or long interval of 15 min) or during certain events (for example: “When I give you a postcard, write your address on it”). Responses were either to be made verbally (e.g. “In 15 minutes, tell me it is time to take a break”) or required an action (e.g. “When I hand you a red pen, sign your name on your paper”). If participants remembered to complete the right task at the right time, they scored 2 points; if they completed the right task but not at the intended time or completed a different task at the specified time, they scored 1 point. Prospective memory was measured as the total sum of these points (range 0 – 48).

**Proactive inhibition**

Proactive inhibition was assessed with a modified Go-NoGo task (Criaud, Wardak, Ben Hamed, Ballanger, & Boulinguez, 2012). For this task, participants were instructed to press the left arrow key on the computer as quickly as possible whenever they saw a big white circle on the screen (“go” target). If a big white cross...
Methods

(“no-go” target) appeared they were instructed to inhibit their response and not press any key. They were also instructed that a small cross in between trials provided information about the next symbol to appear: if the small cross was white, there was a 100% certainty that a white circle was going to be displayed next (“never inhibit” trial). However, if the small cross was red, the next symbol could be either a circle or a cross (“possible inhibition” trial).

While the “possible inhibition” trial required participants to mobilise cognitive resources to prepare for a potential inhibitory response, the “never inhibit” trial did not require such proactive mobilisation. Effective proactive inhibition is therefore indicated by slower response times to the white circle during the “possible inhibition” trials compared to “never inhibit” trials.

The pre-target small cross was presented for either 2000 ms or 6000 ms. Response windows were 1000 ms (with 950 ms target presentation and 50 ms inter-stimulus interval) with 108 “possible inhibition” and 108 “never inhibit” trials. For the “possible inhibition” trials, 48 were “go” trials, 48 were “no-go” trials, and 12 were catch trials, during which no target was presented (to measure commission). For the “never inhibit” trials, 81 were “go” trials and 27 were catch trials.

The dependent variable was measured as the difference in mean response times (in ms) between “possible inhibit” trials and “never inhibit” trials in the “go” conditions.

**Empathic Accuracy**

A modified empathic accuracy task was used to assess how accurately participants were able to infer others’ emotions (Mackes et al., 2018). In brief, participants watched video clips showing a person (referred to here as target) either talking about an emotional event (emotional condition) or describing their bedroom (neutral condition). Participants were asked to continuously rate the target’s emotional
intensity using a 9-point scale (ranging from 1, “no emotion” to 9, “very strong emotion”). Importantly, each video had previously been rated by the targets using the same 9-point scale to assess their continuous emotional intensity throughout the video. Empathic accuracy scores were calculated by correlating the participants’ ratings of the target’s emotional intensity with the target’s own ratings (Pearson’s correlation). These scores were then Fisher z-transformed to assure comparability between correlation coefficients (Fisher, 1915). Participants watched 6 video clips (one female and one male target with one happy, one sad and one neutral video clip each) while lying in the MRI scanner as this was a fMRI task (but only behavioural outcomes of this task will be analysed in this thesis). The dependent variable in this task was the average z-transformed empathic accuracy score which was based on the four emotional (happy and sad) video clips. Higher scores indicated better empathic accuracy.
2.5 **PROCEDURE**

### 2.5.1 Recruitment

Participants were recruited via mail, email and phone and they and their families were invited to come to London to take part in this study. All participants who had been part of the initial sample were contacted unless they or their families had previously stated that they did not wish to further participate. All travel expenses, hotel stays close to King’s College Hospital, and subsistence costs were either reimbursed or booked in advance by the study team to make participation as convenient as possible. Participants received a reminder (by email and phone) one week and one day before the appointment and were reminded to (1) abstain from alcohol and strenuous exercise on the night before the appointment (2) abstain from caffeinated drinks and smoking on the day of the appointment (3) abstain from ADHD-related medication 48 hours before the appointment.

### 2.5.2 Study day

At the beginning of each study appointment, the experimenter and participant read the information sheet together and the participant gave written informed consent to take part. For a copy of the information sheet and consent form see Appendix C and D. The ERABIS protocol involved two MRI scanning sessions, which took approximately one hour each, and were done either on consecutive days or in one day with one scan in the morning and one in the evening. Before each scan, participants were familiarised to the scanning environment in a mock scanning session. During the mock scan, participants lay down in a mock MRI scanner and practised using the mirror by watching a film. They also practised the functional MRI (fMRI) tasks and the use of the button box that was used to record responses. In
most cases, the structural T1 scan was acquired at the beginning of the first
scanning session. Participants were instructed to lie as still as possible during the
scan. During the scanning session, radiographers and experimenter visually
assessed the quality of the scans. In case of substantial movement or other
scanning problems, we repeated the scan. The structural T1 scan was usually
followed by other MRI scans such as a DTI sequence in the first scanning sessions,
and multiple fMRI tasks and scans such as a resting state fMRI scan.

There was also a neuropsychological testing and questionnaire session, which
was organised around the MRI scans and was therefore also either done on
consecutive days or within a single day. The neuropsychological testing included
the MIST, Go-NoGo task and the WASI-II. Tests were performed in semi-
randomised order.

In total, it took about 8 hours for each participant to complete the ERABIS
protocol. Breaks, food and (non-caffeinated) drinks were provided throughout.
Participants were allowed to bring their family members, partner or friends to the
appointment. They were allowed to be in the testing room during the session with
the participant’s consent and the agreement to not disrupt the testing session.
## 2.6 MRI ACQUISITION AND PROCESSING

### 2.6.1 Acquisition

Structural images were acquired on a General Electric MR750 3.0 Tesla MR scanner with a 12-channel head coil. We acquired at least one T1-weighted three-dimensional Magnetization Prepared-Rapid Gradient Echo (MP-RAGE) scan per participant (scanning parameters: TR/TE 7312/3.02ms, flip angle 11°, 256 x 256 matrix, 1.2 mm thick, 196 sagittal slices, FoV=270).

### 2.6.2 Initial quality control

All structural MRI scans were visually inspected. During acquisition, an initial assessment was performed to decide whether the scan needed to be repeated. After the assessment, for each scan, it was recorded (1) whether the whole brain had been scanned, (2) motion artefacts, (3) hypo- and hyper-intensities, (4) ghosting (signal outside of the brain) and (5) other artefacts. If two or more structural T1 scans were available, the higher quality one (usually the last) was chosen for further analysis. All participants who had completed MRI scanning had structural T1 scans suitable for further processing.

### 2.6.3 Processing and further quality control

Cortical thickness (CT), surface area (CSA), volume and local gyrification index as well as subcortical volumes were quantified using FreeSurfer 6.0.0 ([http://surfer.nmr.mgh.harvard.edu](http://surfer.nmr.mgh.harvard.edu)). The procedure has been described in detail elsewhere and will be briefly summarised below (Dale, Fischl, & Sereno, 1999; Fischl et al., 2002; Iglesias et al., 2015; Schaer et al., 2008; Winkler et al., 2012). A visualisation of the processing steps can be found in Figure 2.2. Surface-based
Methods

Analysis with FreeSurfer was chosen over voxel-based morphometry analysis because it allows the differentiation between CT and CSA and gyrification. As these measures have different developmental trajectories (see 1.3.2), it can be hypothesised that they might be affected differentially by early institutional deprivation.

Figure 2.2: Overview of the FreeSurfer processing steps. After surface extraction, the red line indicates the pial surface while the white matter surface is indicated in blue.

2.6.3.1 Surface-based morphometry

First, an affine registration with the MNI305 atlas was performed for each scan. After intensity normalization and skull stripping, white matter was identified by considering each voxels’ intensity values and their neighbours’ constraints. Thereafter, brain stem and cerebellum were “removed”, and hemispheres were “separated”. The white matter surface was generated for each hemisphere using tessellation and then refined by following the intensity gradients between grey and...
white matter. The *pial surface* follows the intensity gradients between grey matter and the CSF. The result is a cortical mesh of triangles for each surface, which consists of about 150,000 vertices (points of triangles) per hemisphere. *CT* was calculated by measuring the closest distance between the white and the pial surface for each vertex. *CSA* was estimated by mapping each vertex into a spherical atlas space and then calculating the average area for each triangle incident to that vertex. *Cortical Volume* was calculated by multiplying CT with CSA at each vertex. In an additional processing step, *local gyrification index* was computed, as described by Schaer et al. (2008). Local gyrification is the ratio between the convoluted inner pial surface and the outer hull surface. In FreeSurfer, the outer visible surface is created first. A circular ROI is then defined on this outer surface and its corresponding area on the pial surface is calculated. Local gyrification index is the ratio of area buried in the sulcus relative to the amount of area on the outer visible layer of the cortex. This step is iterated with overlapping ROIs resulting in a cortical map of gyrification. Higher local gyrification indices indicate higher folding and greater cortical complexity. The different measures are described in Figure 2.3.
Figure 2.3: Surface-based morphometry measures. Cortical thickness is the shortest distance between the white matter and pial surface at each vertex. Cortical surface area is the average area of each triangle incident to a vertex. Local gyrification index is the ratio between inner and outer surface.

2.6.3.2 Volume-based morphometry

The FreeSurfer pipeline also encompasses a volume-based stream, which was used to calculate volumes of subcortical structures. After registration and normalization to the MNI305 atlas and intensity normalization, volumetric labelling was performed based on the intensities of voxels. Hippocampal and amygdala regions were labelled according to a recently developed high resolution atlas which was developed by Iglesias et al. (2015); and Saygin et al. (2017) and is available as an addition to FreeSurfer 6.0 (http://freesurfer.net/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala).

2.6.3.3 Quality control and editing

After the automated surface- and volume-based segmentation, a second quality control step was performed utilising the QA tool script provided by Koh, Lee, Pacheco, Pappu, and Vinke (2012) and updated for FreeSurfer 6.0 usage (https://surfer.nmr.mgh.harvard.edu/fswiki/QATools).
Even though FreeSurfer is a highly robust software package, there can be failures in segmentations. Common failures include: skull strip errors (either brain instead of skull was removed or not enough skull was removed), white matter surface segmentation errors, intensity normalisation errors and pial surface misplacement.

The quality of each segmentation was assessed by first confirming that all processing steps had been completed and all files created. If there were missing files, the required processing steps were repeated. Then, all segmentations were visually assessed using the snapshot tool provided as part of the QA tool. Segmentations that showed failures such as the ones described above were marked for editing. Afterwards, potential segmentation failures were determined by calculating outliers (more than 2 SD from the mean) of the main cortical and subcortical volumes. The signal-to-noise ratio for white matter registration and average white matter intensity was also calculated. Segmentations with outliers, low signal-to-noise-ratios or deviating white matter intensities were inspected more closely for failures.

Overall, I aimed to edit as little as possible to keep reliability of segmentations high. It has been shown that editing has only little impact on the resulting summary measures and might introduce bias in itself (McCarthy et al., 2015). Fortunately, segmentation quality was high throughout and required only minimal editing: For four scans, voxels were manually added to the brain mask as too much had been removed as part of the skull stripping process. For 21 scans, the white matter surface was extended to comprise erroneously excluded white matter due to failed intensity normalisation mostly by changing intensity values of voxels.
2.6.3.4 Pre-processing for statistical analysis and smoothing

In preparation for statistical analysis, every subject was resampled onto a common template, which was based on an average file provided by FreeSurfer (fsaverage 6). Smoothing was performed with a 10 mm kernel at full-width/ half-max (FWHM) for cortical thickness, surface area and volume. As local gyrification index already is a smooth measure (Schaer et al., 2008), only a 5 mm FWHM kernel was applied for smoothing.

2.6.3.5 Statistical analysis

As statistical analysis strategies and multiple comparison correction methods differed for each empirical chapter, they will be discussed in the methods sections of their respective chapters.
2.7 **GROUP COMPARISON RATIONALE**

The overall goal of the current thesis is to examine the relationship between institutional deprivation, brain structure and neurodevelopmental/neuropsychological outcomes. Different approaches to configuring the independent variable (institutional deprivation) are required to optimise the power of the study to address different questions using different statistical approaches - especially given the constraints imposed by the limited sample size available. Where possible effects will be assessed using deprivation as a continuous measure. However, in some analyses it will be necessary to use a categorical approach (e.g. UK vs Rom) especially where statistical interactions between deprivation and symptom levels are being explored. In some cases, especially where the number of UK with symptoms of disorder is low, such analyses will require the combining of the UK and Rom with less than 6 months deprivation into a single group. Below we discuss our decisions in this regard in more detail.

2.7.1 **Chapter 3**

This chapter aimed to investigate the effects of early institutional deprivation on adult brain structure. UK and Rom were compared to investigate whether there was an effect of institutionalisation per se. This was followed by a linear regression with deprivation duration as a continuous variable within Rom to test whether there was a dose-dependent relationship between time spent in the institutions and adult brain structure. This approach was chosen as it is the most powerful and assumption free approach to studying the effects deprivation on brain structure.
2.7.2 Chapters 4 and 5

A different, possibly less powerful, approach was required for Chapter 4 and 5. This was because these chapters focused on the effects of the interaction between deprivation and the presence of symptoms on brain structure. Given the very low levels of symptoms in the UK group it was not possible to meaningfully compare the association between symptoms and brain structure in this group and the Rom group. We decided therefore to combine the UK group with a group within the Romanian sample that could be considered as having low levels of deprivation. This strategy has been used in previous studies to maximize power to explore the interaction between deprivation and other factors (e.g., genetic risk; Kumsta, Stevens, et al., 2010). In those studies, a threshold of 6 months deprivation duration was used to define the boundary of the low deprivation group based on the consistently reported step-wise increase in symptoms at 6 months deprivation duration (Rutter, 1998; Sonuga-Barke et al., 2017). Adoptees exposed to limited deprivation (i.e. institutionalised for less than 6 months; Rom<6) were mostly indistinguishable from UK adoptees in neurodevelopmental problems throughout development. On the other hand, adoptees exposed to extended deprivation (i.e. more than 6 months deprivation duration; Rom>6), were more likely to show a persisting pattern of neurodevelopmental problems (Sonuga-Barke et al., 2017). The Rom<6 group was therefore combined with the UK group to create a Low Deprivation group (LoDep). The same strategy was used in this thesis.

This approach had 2 advantages over its alternatives. First, it was based on a threshold that has strong evidence on a neurodevelopmental symptom level. Second, it allowed all participants to be included in these analyses with at least 40 participants in each group. As mentioned above UK and Rom<6 groups could not be considered separately, because symptom levels in each group were not
sufficient to allow a regression of brain structure on neurodevelopmental symptoms. Furthermore, considering deprivation duration as a continuous variable would have removed all UK adoptees from the analyses in this chapter limiting our ability to draw inferences on brain-behaviour correlates in non-deprived individuals. A LoDep vs Rom>6 was therefore considered the most powerful and meaningful approach for these chapters.

2.7.3 Chapter 6

Chapter 6 aimed to examine the impact of early institutional deprivation on structural covariance of cortical thickness and surface area. As in Chapter 3, we aimed to test the effect of institutionalisation as well as deprivation duration. However, the structural covariance approach is a group-level analysis and individual data is not considered. This means that analyses are limited to group comparisons and cannot allow to investigate continuous variables such as deprivation duration or symptom levels. We decided to compare three groups: adoptees with no deprivation (UK), adoptees with limited deprivation (Rom<6) and adoptees with extended deprivation (Rom>6). Even though we did not consider neurodevelopmental symptoms in this analysis, using a threshold of 6 months deprivation duration was coherent with analyses of the previous chapters and in line with previous ERA reports such as Sonuga-Barke et al. (2017). To confirm that this threshold was as sensitive as other thresholds to examine the effects of deprivation duration on structural covariance, we performed sensitivity analyses moving the threshold to 8, 10 and 12 months.

An overview of the different groupings and their characteristics can be found in Table 2.1.
### Table 2.1: ERABIS participants.

<table>
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<tr>
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<th>sd</th>
<th>median</th>
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<td>0.97</td>
<td>0.21</td>
<td>0.99</td>
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</tbody>
</table>

n – sample size with available data; sd – standard deviation; UK – UK adoptees; Rom<6 – Romanian adoptees with less than 6 months deprivation duration; Rom>6 – Romanian adoptees with more than 6 months deprivation duration
2.8 SELECTIVE ATTRITION ANALYSIS

2.8.1 Rationale

Longitudinal studies allow us to investigate causal effects in developmental processes and trajectories (Beaver, 2013). Attrition is one of the biggest concerns in longitudinal designs as it reduces sample sizes over time, and it can introduce bias in the case of selective attrition (i.e. if populations or individuals with certain characteristics are more likely to drop out; Nicholson, Deboeck, & Howard, 2015). Previous ERA follow-ups had low attrition with 91% and 75% of the original sample providing at least some data at the age 15 and young adulthood follow-ups, respectively (Rutter et al., 2012; Sonuga-Barke et al., 2017). In comparison, retention in ERABIS was considerably lower. This is likely due to multiple factors. For example, while ERA young adult follow-up researchers visited participants at home, MRI scanning as part of ERABIS required travelling to London (in some cases, this involved a journey of thousands of miles). Moreover, some individuals were not able to be scanned, e.g. if they had had operations that involved the insertion of metal plates or pins. ERA participants and their families have shown great commitment to, and involvement in, this study. ERABIS tried to retain as much of the sample as possible, e.g. by covering travel expenses of participants travelling from as far away as New Zealand. This resulted in a retention rate of approximately 40%. While previous follow-ups have shown no signs of selective attrition, this should also be ascertained for ERABIS in order to confirm the generalisability of our results.
2.8.2 Methods

For this selective attrition analysis, individuals who took part in ERABIS and were included in the analyses (n=88) were compared to individuals who had dropped out (i.e. did not take part in ERABIS or could not be included in the analyses; n=129). Independent t-tests were applied to test for group differences in deprivation duration, IQ and ADHD, ASD and DSE symptoms at age 6 (see 2.4.2.1). Age 6 data (the first full assessment wave) were used to ascertain that data were available for most of the participants. We first compared ERABIS participants and drop-outs as a whole and then split groups into UK, Rom<6 and Rom>6 to examine if any of the groups were particularly affected by attrition.

2.8.3 Results

There was no difference in deprivation duration, ADHD symptoms, ASD symptoms, DSE symptoms and IQ as measured at age 6 between individuals who took part in ERABIS compared to those who had dropped out (all ps>0.08). When examining UK, Rom<6 and Rom>6 separately, there was no indication for selective attrition in the UK and Rom<6 groups (all ps>0.24). However, in the Rom>6 group, individuals who had been included in ERABIS had significantly lower ASD symptoms compared to those who had dropped out (t(96)=2.89, p<0.01, Figure 2.4).
Methods

Figure 2.4: Selective attrition analysis. For each neurodevelopmental problem and group, swarm- and point-plots explore the difference between drop-outs and individuals included in ERABIS. Black dots and whiskers represent mean values and their 95% confidence intervals. Dots represent individual data points. Top row: ADHD symptoms, second row: ASD symptoms, third row: DSE symptoms, fourth row: IQ. UK adoptees in red (left), Rom<6 in purple (middle), Rom>6 in orange (right). Significant p-values are marked in bold.
2.8.4 Conclusion

In line with previous ERA follow-ups, there was no strong evidence for selective attrition in ERABIS. However, in the highly deprived group, individuals with a higher ASD symptoms, were more likely to have dropped out. The same effect was not seen in UK and Rom<6, maybe because they have lower rates of ASD symptoms. The findings for the other neurodevelopmental domains did not provide any evidence for selective attrition. The results of this study can therefore still be considered generalisable to the broader ERA sample with regard to symptoms of ADHD and DSE and IQ. However, the selective drop-out of individuals with high ASD symptoms following extended deprivation will restrict our ability to study the relationship between deprivation, ASD symptoms and brain structure and the effects of deprivation on ASD symptoms might be underrepresented.
2.9 ACCURACY OF AUTOMATED SEGMENTATION ANALYSIS

2.9.1 Rationale

Hippocampus and amygdala are hypothesised to be core areas sensitive to early maltreatment (Teicher, Samson, Anderson, & Ohashi, 2016). However, the results of previous studies examining the effects of early maltreatment (and specifically early institutional deprivation) have been mixed. A potential reason for inconsistencies might be measurement error of different segmentation approaches (McCropy et al., 2010). To be able to investigate the effect of institutional deprivation on amygdala and hippocampus, it was of utmost importance for our study to accurately and reliably identify and quantify these brain regions. Until recently, manual segmentation of these structures was considered the gold standard for volumetric quantification (Boccardi et al., 2011; Schmidt et al., 2018). However, manual tracing is very time consuming, requires anatomical expertise and a consistent approach with segmentation protocols (Schoemaker et al., 2016). There is also a potential of introducing experimenter bias. Therefore, neuroimaging studies often employ automated segmentation approaches for a volumetric quantification of these brain structures. These automated protocols are fast, show high reproducibility and do not require very extensive anatomical knowledge of the user (Schoemaker et al., 2016). The most commonly used software packages for automated segmentation are FreeSurfer and FSL FIRST (Morey et al., 2009). Schoemaker et al. (2016) found that FreeSurfer performs better than FIRST in segmenting amygdala and hippocampus but even FreeSurfer segmentations showed only moderate correlation with manual tracing of the amygdala ($r=0.56$). The recently released FreeSurfer high resolution atlas segmentation (Iglesias et al., 2015; Saygin et al., 2017) showed the highest accuracy compared to manual
tracing of hippocampal volumes (Schmidt et al., 2018). However, no study has yet investigated accuracy of amygdala segmentations using the new atlas. In this analysis, the new FreeSurfer high resolution atlas segmentations were compared to manual tracing of a subset of amygdala and hippocampal volumes in our sample. This analysis aimed to test whether the automated segmentation accuracy was satisfactory to be used instead of manual segmentation in the whole sample.

2.9.2 Methods

An expert in manual segmentation (rater 1) trained another member of the study team (rater 2) and me (rater 3) to manually segment hippocampal and amygdala volumes. All raters followed well established segmentation protocols for amygdala (Center for Neuroscience and the M.I.N.D. Institute, 2003) and hippocampal volumes (Boccardi et al., 2015). The protocols can be found in Appendix E and F. The software ITK-SNAP (Version 3; Yushkevich et al., 2006) was used to manually assign voxels to each of the volumes (left amygdala, right amygdala, left hippocampus, right hippocampus). Training was performed on two brain scans from our sample which had been chosen at random. After training, 8 brain scans were randomly selected from our sample, in addition to 8 brain scans from healthy volunteers (aged between 20 and 30 years, with no current or recurrent mental illness) which had been acquired on the same scanner using the same scanning parameters. Left and right amygdala and hippocampal volumes of these 16 participants (10 females) were automatically segmented with the FreeSurfer 6.0 high resolution atlas extension (as described in 2.6.3.2). Rater 1 manually segmented left and right hippocampal volumes of 6 brain scans; rater 2 segmented left and right amygdala volumes of all 16 scans, and rater 3 segmented left and right amygdala and hippocampal volumes of all 16 scans. Raters were blinded to group
belonging and the results of other raters’ manual and automated segmentations.

Intraclass-correlation coefficients (ICC, two-way mixed, consistency) were used to compare the manual and automated segmentations (Koo & Li, 2016). This was done for each anatomical region and hemisphere separately, and with left and right volumes combined. The threshold for acceptable reliability was an ICC of 0.70.

2.9.3 Results

Manual segmentations showed good to excellent inter-rater reliability (Table 2.2). FreeSurfer 6 performed acceptably for both amygdala and hippocampal volumes in comparison to manual segmentations. On average, FreeSurfer 6 overestimated hippocampal volumes by 22% and underestimated amygdala volumes by 4% compared to manual segmentation.

Table 2.2: Comparison of manual and automated segmentation. Intraclass correlation coefficients (two-way mixed, consistency) between rater 3 (who performed segmentations of the complete subset) and rater 1, rater 2 and FreeSurfer 6 segmentation results.

<table>
<thead>
<tr>
<th></th>
<th>rater 1</th>
<th>rater 2</th>
<th>FreeSurfer 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>left amygdala</td>
<td>0.85</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=16)</td>
<td></td>
</tr>
<tr>
<td>right amygdala</td>
<td>0.91</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=16)</td>
<td></td>
</tr>
<tr>
<td>combined measures</td>
<td>0.88</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=32)</td>
<td>(n=32)</td>
<td></td>
</tr>
<tr>
<td>left hippocampus</td>
<td>0.90</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=16)</td>
<td></td>
</tr>
<tr>
<td>right hippocampus</td>
<td>0.98</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=16)</td>
<td></td>
</tr>
<tr>
<td>combined measures</td>
<td>0.94</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=12)</td>
<td>(n=32)</td>
<td></td>
</tr>
</tbody>
</table>

n – sample size

2.9.4 Conclusion

Good to excellent reliability between manual segmentations demonstrates the successful application of the protocols. Good accuracy of FreeSurfer segmentations seemed acceptable considering its many advantages over manual tracing. Not only is the automated segmentation much more time efficient (manual segmentation took
one hour per scan on average), FreeSurfer also offers consistently high accuracy with no risk of bias or fatigue. FreeSurfer segmentations of amygdala and hippocampus were therefore used for all further analyses.
2.10 Overview of Future Chapters

This chapter described the study design, including recruitment, data collection and pre-processing of neuroimaging data. It also examined selective attrition in our sample and accuracy of the automated segmentation of amygdala and hippocampal volumes. The following empirical chapters set out to answer how early childhood institutional deprivation alters adult brain structure and structural covariance. Chapter 3 will explore the effects of deprivation on total brain volume and regional differences in cortical thickness, surface area, volume and gyrification. Chapter 4 will examine the neurodevelopmental and neuropsychological correlates of these brain structural alterations. Chapter 5 will test on the whole-brain level, whether brain structural correlates of deprivation-related neurodevelopmental symptoms are distinct compared to non-deprivation-related symptoms. Finally, Chapter 6 will explore whether institutional deprivation is associated with alterations in structural covariance.
CHAPTER 3

IMPACT OF EARLY INSTITUTIONAL DEPRIVATION

ON ADULT BRAIN STRUCTURE
ABSTRACT

Early childhood deprivation is associated with severe and long-lasting neurodevelopmental impairment, yet the associated impact on brain development is currently unknown. We used magnetic resonance imaging to examine brain structure in young adults who had lived in severely depriving institutions in Romania during the Ceaușescu era until they were adopted into nurturing families as young children. This prospective natural experimental design strengthens causal inferences on the association between early deprivation and adult brain structure. Sixty-seven Romanian adoptees, who had lived between 3 and 41 months in conditions of severe deprivation in the institutions, were compared to 21 non-deprived UK adoptees. Romanian adoptees had substantially smaller brains than non-deprived adoptees – an effect that increased in a linear fashion as a function of deprivation duration. This effect could not be explained by other factors such as overall smaller body height. Beyond such global effects, there were localized deprivation-related effects with smaller surface area and volume in the right inferior frontal cortex, and bigger surface area, thickness and volume in the right inferior temporal cortex. Moreover, deprivation duration was positively associated with surface area and volume in the right medial prefrontal cortex. Subcortical volumes such as amygdala and hippocampus were not associated with deprivation after controlling for total brain volume.

Together these findings show that – more than 20 years after it has ended – early institutional deprivation is associated with global and regional alterations in brain structure, which most likely represent a causal and neurobiologically distinct effect.
3.1 INTRODUCTION

Neuroplasticity, the brain’s inherent ability to dynamically adapt and change in response to environmental influences, supports normal learning and development and allows recovery of function following injury (Zatorre, Fields, & Johansen-Berg, 2012). At the same time, it is hypothesized to leave the brain vulnerable to the effects of adverse psychosocial experiences such as maltreatment (Stevens & Neville, 2006). This is thought to be especially true during sensitive periods of development in early life when the brain is particularly malleable (Ismail et al., 2017) – in ways that may increase the risk of mental disorders later in life (Teicher & Samson, 2016). This could either be because exposure to experiences thought necessary for normal development does not occur (a failure in ‘experience-expectant’ programming) or because an organism’s brain adapts in anticipation of adversity in the future (reflecting ‘experience-adaptive’ programming) (Rutter & O'Connor, 2004). Studies using experimental animal models of maltreatment provide support for these hypotheses (Weaver, Meaney, & Szyf, 2006) especially in relation to the amygdala, hippocampus and prefrontal cortex - perhaps because of their close links to the hypothalamus-pituitary-adrenal axis (Lupien et al., 2009; Morimoto et al., 1996). However, evidence for early maltreatment effects on brain structure from human studies, which for ethical reasons cannot experimentally manipulate exposure to adversity, is often difficult to interpret. This is because of design limitations that restrict the ability to infer a causal role for the environmental exposure (Rutter et al., 2012). For instance, studies of the effects of maltreatment by biological parents are potentially confounded in several important ways. Common genetic factors may drive both the abusive behaviours of the maltreating parent and the brain development of the maltreated child (Dinkler et al., 2017). Furthermore, reliance on retrospective accounts of adverse exposures based on
Impact of early deprivation on adult brain structure

parental or self-report reduce confidence in the veracity of early maltreatment status and lead to possible oversampling of individuals suffering from psychopathology (Hardt & Rutter, 2004; Newbury et al., 2018; Reuben et al., 2016). This makes it difficult to isolate with any certainty the effects of early adversity on the brain from brain-based manifestations of psychopathology (Teicher & Samson, 2016).

Moreover, individuals who experience maltreatment early in life are also likely to experience it throughout later development (Dunn et al., 2011), which is problematic when trying to draw inferences on sensitive periods of development.

Prospective longitudinal studies of children exposed to time-limited deprivation within non-familial institutions - rather than in the biological family - and then adopted into normal loving and nurturing families offer the best opportunity to study the effects of early adverse environmental exposures on brain development in a design with reduced risk of confounding by pre-existing genetic factors or ongoing adversity. Inference about the causal role of the adverse exposure is strengthened further when the switch from deprived to enriched environment is abrupt, precisely timed, and unlikely to be determined by underlying risk within the child rather than other circumstances (Rutter et al., 2012). The large-scale international adoption of the young children found living in the appalling conditions of the Romanian orphanages at the time of the fall of the Ceaușescu regime represents an example of such a natural experiment.

To date, most studies of this cohort have focused on cognitive and mental health outcomes rather than brain development - concluding that extended deprivation is associated with increased rates of neurodevelopmental and mental disorders that are often persistent in nature (Sonuga-Barke et al., 2017; Zeanah et al., 2009). The handful of studies that have examined brain structure using magnetic resonance imaging (MRI) provide evidence for an association between
early institutional deprivation and reduced grey and white matter volumes in childhood and early adolescence (Hanson et al., 2013; Hanson et al., 2015; Hodel et al., 2015; McLaughlin, Sheridan, Winter, et al., 2014; Mehta et al., 2009; Sheridan et al., 2012; Tottenham et al., 2010). Results are, however, inconsistent with regard to the regional specificity of effects. There is evidence for smaller volumes in the prefrontal cortex (Hodel et al., 2015) and cortical thinning in prefrontal, parietal and temporal regions (McLaughlin, Sheridan, Winter, et al., 2014). Findings on amygdala and hippocampal volumes are inconsistent however (Hanson et al., 2015; Hodel et al., 2015; Mehta et al., 2009; Sheridan et al., 2012; Tottenham et al., 2010).

The English and Romanian Adoptees (ERA) Study is the first to investigate whether the effects of this form of early institutional deprivation on brain structure endure into young adulthood. The ERA study examined Romanian adoptees who had entered the institutions in the first few weeks of life and then spent between 2 weeks and 41 months living in severe deprivation before being adopted into families in the UK that mainly provided a well-functioning, nurturing environment. The study also included a comparison group of non-deprived UK adoptees placed before 6 months of age (Rutter, 1998). Initial reports documented a devastating initial effect of deprivation on development followed by subsequent rapid recovery by the age of 6 years (Rutter, 1998). Despite these improvements, many individuals who spent an extended period (i.e., >6 months) in the institutions subsequently displayed a strikingly persistent and impairing pattern of neurodevelopmental disorders including attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD) and disinhibited social engagement (DSE; indiscriminate friendliness towards strangers and lack of selectivity in attachment-related behaviours; American Psychiatric Association, 2013) (Kennedy et al., 2016; Sonuga-Barke et al., 2017).
Impact of early deprivation on adult brain structure

We employed a comprehensive whole-brain approach including surface-based morphometry and subcortical volume analysis to systematically investigate how early institutional deprivation impacts global brain volume and local brain structure in early adult life.

First, we examined whether institutionalisation and deprivation duration were associated with total brain volume. Moreover, we tested whether changes in global brain volume indicated a neurobiological rather than general physical growth effect: we explored the relationship between total brain volume and overall body height (which has been shown to be affected by institutional deprivation in this sample, Rutter et al., 2007). To do so, we first analysed the relationship between these measures in a large data-set of non-adopted adults and the resulting model was used as a norm for the Romanian adoptees group. Next, in order to strengthen causal inferences, we tested whether the effects on total brain volume could be better explained by pre-existing factors such as genetic risk for smaller intracranial volumes or birth weight (as an indicator for prenatal environment) rather than by the deprivation experience. To gain a further understanding of the aspects driving the deprivation effects, we examined if total brain volume was predicted by subnutrition (as indicated by weight at adoption).

Furthermore, we used a whole brain approach to test if - over and above total brain volume – institutionalisation and deprivation duration were related to local alterations in specific brain regions examining cortical surface area, thickness, volume and gyrification. We also tested whether these we correlated with weight at adoption as a marker for subnutrition in the institutions.

Finally, we tested, whether subcortical volumes, including limbic volumes of amygdala and hippocampus, basal ganglia volumes of putamen, caudate, pallidum,
Impact of early deprivation on adult brain structure

and accumbens and thalamus volumes could be predicted by institutionalisation or deprivation duration.

We made a number of predictions based on the following sets of hypotheses.

1) Global alterations following institutional deprivation

We hypothesised that institutionalisation would be associated with global reductions in adult brain volumes: Romanian adoptees would have smaller total brain volume compared to UK adoptees. Furthermore, we hypothesised duration of deprivation to contribute to this effect in a dose-dependent way and therefore predicted a negative linear relationship between brain volume and deprivation duration. Finally, as there is some indication for potential recovery of total white matter volumes following institutional deprivation (Sheridan et al., 2012), we predicted that deprivation effects might be stronger in grey compared to white matter volumes.

2) Factors underlying global alterations in brain volume

We hypothesised that alterations in total brain volume would indicate a specific neurodevelopmental effect rather than simply being the result of overall growth stunting following deprivation. We therefore predicted that the effect of deprivation duration on total brain volume could not be explained by overall smaller body height - total brain volume would be disproportionately affected by deprivation. Furthermore, we predicted that effects of institutionalisation on total brain volume could not be explained by other pre-existing genetic or prenatal factors as indicated by polygenic scores for intracranial volume and birth weight.

3) Regional alterations in brain structure

Based on previous animal and human studies on early life stress (Hodel et al., 2015; Lupien et al., 2009; Teicher & Samson, 2016), we hypothesised that the prefrontal cortex, due to its protracted postnatal development, would be particularly sensitive to institutional deprivation. We therefore predicted that, over and above
this global effect, prefrontal brain regions would be negatively associated with institutionalisation and deprivation duration.

We hypothesised that cortical thickness, surface area and local gyrification (folding of the cortex) would show differential effects of institutionalisation as they follow different developmental trajectories (Li et al., 2014; Lyall et al., 2015). Cortical surface area is less established at birth compared to cortical thickness and local gyrification (Li et al., 2014; Lyall et al., 2015), which might make it more sensitive to environmental effects such as institutional deprivation. Therefore, we predicted stronger and more widespread differences in cortical surface area.

4) Subcortical alterations

Based on previous institutional deprivation and maltreatment research, it was unclear whether limbic structures of amygdala and hippocampus would show volumetric differences following deprivation. Animal models and human observational studies on early childhood maltreatment consistently report smaller hippocampal volumes but inconsistent findings for amygdala volumes (Lupien et al., 2009; McCrory et al., 2010; Teicher & Samson, 2016). The only other study that investigates the effects of institutional deprivation in a cohort of Romanian adoptees has not found subcortical volumetric differences in childhood (Sheridan et al., 2012), while our own pilot study found larger amygdala volumes but no differences in hippocampal volumes in adolescence (Mehta et al., 2009). Other subcortical structures have seldom been investigated, with some indication of putamen volumes being sensitive to maltreatment (see 1.4.3.2). We therefore had no hypothesis whether and in what direction these structures would show differences in young adulthood.
3.2 METHODS

3.2.1 Participants

Participants were recruited as described in 2.3 and 2.5.1, and the final sample comprised 67 Romanian adoptees (Rom; 40.6% of the original sample, 50.7% female, mean age=25.3 years, SD age=1.1 years,) and 21 UK adoptees (UK, 40% of the original sample, 38.1% female, mean age=24.4 years, SD age=1.0 years). For the Rom group, deprivation duration ranged between 3 and 41 months.

3.2.2 Measures

Measures are described in detail in 2.4. The following measures were included in this chapter.

3.2.2.1 Physiological measures

Young adult body height (in cm), head circumference (in cm) and polygenic scores for intracranial volume were measured during ERA young adult follow-up. Subnutrition (in SD of UK norms) was recorded at UK entry. Birth weight was based on Romanian records.

3.2.2.2 Human Connectome Project

To investigate whether alterations in total brain volumes were the result of smaller body height following institutionalisation, we aimed to characterise the relationship between both measures in a large data-set of young adults. To do so, we analysed data from the Human Connectome Project (Van Essen et al., 2012). Measures of body height (provided in inches, converted into cm), total brain volume, and gender were available for 716 participants aged between 20-30 years (48.6% female, mean age=26.6 years, SD=2.5 years). Total brain volumes were based on structural T1-
weighted scans collected at the same magnet strength as in our study (3 Tesla), analysed and segmented in FreeSurfer 5.3.0.

3.2.3 Procedure, MRI data acquisition and processing

Please refer to 2.5 and 2.6 for a detailed description. In brief, a structural T1 scan was acquired on a 3.0 Tesla MR scanner with a 12 channel head coil as part of the ERABIS protocol. Cortical thickness, surface area, volume and local gyrification index as well as subcortical volumes were quantified using FreeSurfer 6.0.0. Smoothing was performed with a 10 mm kernel at full-width/half-max (FWHM) for cortical thickness, surface area and volume, and a 5 mm FWHM for local gyrification.

3.2.4 Statistical analysis

3.2.4.1 Descriptive statistics

Analyses were performed in R 3.5.0 (R Core Team, 2018). We tested for differences between UK and Rom groups in young adult head circumference, body height and polygenic scores for intracranial volumes using general linear models (controlling for sex as a covariate).

3.2.4.2 Global alterations following institutional deprivation

To test the first set of hypotheses, general linear models were used to test for differences between UK and Rom groups in total brain volume (TBV), total grey and white matter volumes. Furthermore, linear regressions were performed within the Rom group to test whether these measures correlated with deprivation duration when employed as a continuous measure. Sex was entered as a covariate in all analyses, as sex differences in brain volume are well-established (Ruigrok et al., 2014).
3.2.4.3 Factors underlying global alterations in brain volume

In order to investigate the second set of hypotheses, in separate general linear models, body height and head circumference were added as covariates, to test whether deprivation duration predicted TBV after controlling for these factors. To identify the relationship between body height and total brain volume in the general population, Human Connectome Project data were used to predict TBV based on height, while controlling for gender as a covariate. The resulting regression parameters were used to predict TBV based on height in our Rom group. We compared these with observed TBV in a repeated measures ANOVA.

In subsequent analyses, we used linear regressions to test whether deprivation duration predicted birth weight and polygenic scores of intracranial volume. We also tested whether deprivation duration predicted TBV if additionally controlling for polygenic scores of intracranial volume or birth weight as a covariate and if TBV was predicted by subnutrition (weight at UK entry).

Again, sex was entered as a covariate in all analyses.

3.2.4.4 Regional alterations in brain structure

To test for regional alteration beyond global effects, in a whole-brain approach, we first tested for differences between UK and Rom groups in cortical thickness, surface area, volume and local gyrification using general linear models. Second, whole-brain linear regression analyses were performed within the Rom group to investigate if there was a linear relationship between duration of deprivation and any of these measures. Third, we tested whether these changes were related to subnutrition in linear regressions (with the average measure per vertex for each significant cluster as dependent variables). These analyses were performed with FreeSurfer 6.0.0.
In addition to sex, TBV was entered as a covariate for volume, surface area and local gyrification measures (as these, but not cortical thickness, scale closely with TBV; Barnes et al., 2010) to examine whether there were regional differences between the groups which were not proportional to global brain volume. For all whole brain analyses, cluster-wise correction for multiple comparisons was performed using a Monte Carlo simulation (vertex-wise threshold $p<.05$, clusterwise-threshold $p<.05$).

### 3.2.4.5 Subcortical alterations

We tested for differences between the UK and Rom groups in relative subcortical volumes (including sex and TBV as covariates) in a multivariate general linear model. The volumes examined were the amygdala, hippocampus, thalamus, nucleus accumbens, caudate nucleus, putamen and pallidum for the left and right hemisphere separately. Furthermore, partial correlations were performed to identify if deprivation duration or subnutrition were related to relative subcortical volumes (controlling for sex and TBV). All analyses were corrected for multiple comparisons using the False Discovery Rate procedure (FDR; $q=0.05$; Benjamini & Hochberg, 1995).
3.3 RESULTS

3.3.1 Descriptive statistics

Compared to the UK group, Rom showed significantly smaller head circumference and body height in young adulthood, while the difference in polygenic scores for intracranial volume was not significant (Table 3.1).

Table 3.1: Descriptive statistics for UK and Rom groups. Significant group differences are marked in bold.

<table>
<thead>
<tr>
<th>measure</th>
<th>group</th>
<th>n</th>
<th>mean</th>
<th>sd</th>
<th>median</th>
<th>range</th>
<th>group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>deprivation duration</td>
<td>UK</td>
<td>67</td>
<td>16.19</td>
<td>NA</td>
<td>15.00</td>
<td>38.00</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Rom</td>
<td>57</td>
<td>16.71</td>
<td>10.98</td>
<td>15.00</td>
<td>38.00</td>
<td>NA</td>
</tr>
<tr>
<td>birth weight [kg]</td>
<td>UK</td>
<td>60</td>
<td>2.76</td>
<td>0.68</td>
<td>2.90</td>
<td>3.84</td>
<td>F(1,75)=21.96,</td>
</tr>
<tr>
<td></td>
<td>Rom</td>
<td>58</td>
<td>2.58</td>
<td>0.68</td>
<td>2.90</td>
<td>3.84</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>weight at UK entry [SD from UK norms]</td>
<td>UK</td>
<td>58</td>
<td>-2.32</td>
<td>1.81</td>
<td>-2.41</td>
<td>8.66</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Rom</td>
<td>57</td>
<td>-2.18</td>
<td>1.81</td>
<td>-2.41</td>
<td>8.66</td>
<td>NA</td>
</tr>
<tr>
<td>young adult head circumference [cm]</td>
<td>UK</td>
<td>21</td>
<td>57.85</td>
<td>1.73</td>
<td>58.00</td>
<td>5.77</td>
<td>F(1,75)=45.20,</td>
</tr>
<tr>
<td></td>
<td>Rom</td>
<td>57</td>
<td>55.67</td>
<td>1.90</td>
<td>55.50</td>
<td>8.07</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>young adult body height [cm]</td>
<td>UK</td>
<td>21</td>
<td>177.55</td>
<td>8.99</td>
<td>177.50</td>
<td>34.70</td>
<td>F(1,75)=45.20,</td>
</tr>
<tr>
<td></td>
<td>Rom</td>
<td>57</td>
<td>164.09</td>
<td>9.25</td>
<td>163.83</td>
<td>45.50</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Polygenic scores for intracranial volumes</td>
<td>UK</td>
<td>16</td>
<td>-0.05</td>
<td>0.04</td>
<td>-0.06</td>
<td>0.15</td>
<td>F(1,62)=3.20,</td>
</tr>
<tr>
<td></td>
<td>Rom</td>
<td>49</td>
<td>-0.08</td>
<td>0.06</td>
<td>-0.07</td>
<td>0.23</td>
<td>p=0.08</td>
</tr>
</tbody>
</table>

n – sample size with data available; sd – standard deviation; UK – UK adoptees group; Rom – Romanian adoptees group

3.3.2 Global alterations following institutional deprivation

Institutionalisation was associated with a 8.57% reduction in TBV (F(1,85)=20.55, p<0.001, SE=21.99, Cohen’s d=-1.13, Figure 3.1a) and deprivation duration was a strong negative predictor of TBV in the Rom group (β=-0.31, r_{partial}=-0.41, t(64)=-3.62, p<0.001, Figure 3.1b): Each additional month of deprivation was associated with a 3.00 cm³ (0.28%) reduction in TBV by young adulthood. Results were similar for total grey and white matter volumes (Figure 3.1c-f). For all linear models, outcome variables were normally distributed (all Shapiro-Wilk tests (SW)>0.98, ps>0.21).
Figure 3.1: Deprivation-related differences in total brain, grey and white matter volumes. Point- and swarm-plot depicting the distributions of total brain volume (TBV, a), grey matter volume (c) and white matter volume (e) in the UK and Romanian adoptees (Rom). Black whiskers show 95% confidence intervals around the means (black dots). Negative correlation between deprivation duration and TBV (b), grey matter volume (d) and white matter volume (f). The shaded area depicts the 95% confidence interval around the regression line. The effect of sex has been regressed out for all volumes.
3.3.3 Factors underlying global alterations in brain volume

These effects were not simply a reflection of more general deprivation-related reductions in overall growth - which was also extremely common in our sample (Rutter et al., 2007). Deprivation duration was still a significant predictor for TBV in Rom after controlling for body height and head circumference. An analysis of 716 young adults in the Human Connectome Project (Van Essen et al., 2012), revealed that in the general population body height and TBV show a small but significant association when controlling for sex as a covariate ($\beta=0.28$, $r_{\text{partial}}=0.25$, $t(713)=6.83$, $p<0.001$, Figure 3.2). Using these regression parameters, we estimated that TBV in the Rom group was on average still 7.76% smaller than the volumes that would be predicted by height alone ($F(1,55)=168.04$, $p<0.001$) – suggesting that brain growth was disproportionately affected by early deprivation compared to overall growth.

**Figure 3.2: Scatterplot of correlation between body height and total brain volume.**
There was a small positive association in 716 young adults of the Human Connectome Project (HCP). In comparison, Romanian adoptees tend to have smaller TBV and body height and TBV is even smaller than would be expected based on height. Sex has been regressed out for both TBV and body height.
There was no evidence that those exposed to greater institutional deprivation were at increased genetic risk (as indexed by a polygenic score for intracranial volume; $\beta=0.08$, $t(46)=0.58$, $p=0.56$, Figure 3.3a) or adversity experienced prenatally (as indexed by birth weight; $\beta=0.12$, $t(55)=0.90$, $p=0.37$, Figure 3.3b) and controlling for these factors did not alter the results. Subnutrition does not appear to have played a role in these profound and long-lasting effects on brain size – as TBV was unrelated to weight at time of adoption ($\beta=0.19$, $t(57)=1.91$, $p=0.07$, Figure 3.3c), although the composition of diet was not measured.

![Figure 3.3](image)

**Figure 3.3: Possible influences for smaller total brain volume and deprivation duration.** Neither birth weight (a) nor polygenic risk scores (b) were related to how long adoptees spent in the institutions. c) Subnutrition during institutionalisation did not predict adult TBV.

### 3.3.4 Regional alterations in brain structure

#### 3.3.4.1 Effects of institutionalisation per se

Institutionalisation was associated with: (i) significantly smaller surface area and volume in the right inferior frontal gyrus (pars triangularis) extending into the rostral middle frontal gyrus and (ii) significantly greater cortical surface area, thickness and volume in a cluster extending from the right inferior temporal gyrus into the anterior fusiform gyrus, parahippocampus and temporal pole (Figure 3.4, Table 3.2). These regional changes did not vary as a function of deprivation duration or subnutrition.
3.3.4.2 Effects of deprivation duration

As it was unclear whether structural brain changes would follow the same duration-related effects as seen in the neurodevelopmental symptom domains (i.e., with a step increase in risk after around 6 months of deprivation), we also conducted a linear regression to test for a dose-response relationship between deprivation duration and regional cortical measures in the Rom group separately. Such effects were largely absent although deprivation duration was positively correlated with surface area and volume in the right superior frontal cortex, extending to the right medial orbitofrontal cortex and rostral anterior cingulate cortex (Figure 3.4, Table 3.2).
Impact of early deprivation on adult brain structure

Figure 3.4: Deprivation-related regional differences in cortical volume, thickness and surface area. (i) Romanian adoptees had smaller surface area and volume in a cluster in the right inferior frontal gyrus. (ii) Romanian adoptees had bigger thickness, surface area and volume in a cluster in the right inferior temporal gyrus. (iii) Significant correlation between deprivation duration and surface area and volume of a cluster in the right superior frontal, medial orbitofrontal and anterior cingulate cortex. Brain maps are displayed on the left. Point- and swarm-plots on the right display averages of vertex-wise measures of each cluster with dots representing individual participants. Black whiskers show 95% confidence intervals around the mean. All clusters were significant on a whole brain level corrected for multiple comparisons (clusterwise-threshold $p < .05$). Effect sizes ($Cohen’s d$ and Pearson’s $r$) of each cluster were derived from whole-brain vertex-wise effect size brain maps. All analyses controlled for TBV (except cortical thickness analyses) and sex as covariates. Individual data points represent measures after regressing out these covariates.
Table 3.2: Clusters showing significant differences in cortical volume, surface area, thickness or local gyrification. Monte Carlo correction for multiple comparisons was applied (clusterwise-threshold p<.05). Effect sizes (Cohen’s d and Pearson’s r) were taken from whole-brain vertex-wise effect size brain maps.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Anatomical region</th>
<th>H</th>
<th>Cluster size [mm²]</th>
<th>Peak MNI coordinates [mm]</th>
<th>Cluster-wise p</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UK &gt; Rom</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>inferior frontal</td>
<td>R</td>
<td>1269</td>
<td>55</td>
<td>17</td>
<td>0.0004</td>
</tr>
<tr>
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<td>rostral middle</td>
<td>R</td>
<td>1859</td>
<td>42</td>
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</tr>
<tr>
<td><strong>UK &lt; Rom</strong></td>
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</tr>
<tr>
<td>Volume</td>
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<td>R</td>
<td>800</td>
<td>52</td>
<td>-26</td>
<td>0.0331</td>
</tr>
<tr>
<td>Area</td>
<td>inferior temporal</td>
<td>R</td>
<td>1708</td>
<td>44</td>
<td>-17</td>
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<tr>
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<td>inferior temporal</td>
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<td>1178</td>
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<td>-27</td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Volume</td>
<td>superior frontal</td>
<td>R</td>
<td>1252</td>
<td>10</td>
<td>63</td>
<td>0.0004</td>
</tr>
<tr>
<td>Area</td>
<td>superior frontal</td>
<td>R</td>
<td>2721</td>
<td>14</td>
<td>46</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

H: hemisphere; L: left; R: right; r<sub>partial</sub>: partial correlation coefficient

3.3.5 Subcortical alterations

Previous literature, including a pilot brain imaging study embedded in an earlier wave of ERA (Mehta et al., 2009), had provided important initial evidence that the limbic system is particularly vulnerable to maltreatment and institutional deprivation (Teicher & Samson, 2016). Here, however, we found that, over and above TBV effects, there was no effect of institutional deprivation on limbic regions such as the amygdala and hippocampus or other subcortical structures including the thalamus, nucleus accumbens, caudate, putamen and pallidum (Rom vs. UK: all p<sub>FDR</sub> > 0.40; duration of deprivation effects: all p<sub>FDR</sub> > 0.65, Figure 3.5). Subnutrition (as indexed by weight at time of adoption) did not predict subcortical volumes (all p<sub>FDR</sub> > 0.94).
Figure 3.5: Subcortical volumes. For illustration purposes, the average volume of left and right structures for each individual is displayed while analyses were performed for each structure and hemisphere separately. From left to right, up to down, the subcortical volumes are: amygdala, hippocampus, nucleus accumbens, caudate, putamen, pallidum and thalamus. Upper panel of each volume: there were no significant group differences between UK adoptees and Romanian adoptees in subcortical volumes. Whiskers represent 95% confidence intervals around the mean (black dot). Lower panel of each volume: Deprivation duration was not correlated with subcortical volumes. All analyses controlled for TBV and sex and individual data points represent volumes after regressing these covariates.
3.4 DISCUSSION

3.4.1 Global alterations following institutional deprivation

We provide the strongest evidence to date of the power of time-limited severe early adversity to determine long-term brain development leading to substantially smaller global brain volume in early adulthood – more than twenty years after adoptees left the institutions and were adopted into supportive and nurturing families. Not only did young adults who had experienced deprivation have substantially smaller brains than non-deprived UK adoptees but within the Romanian adoptee group there was a strong and linear negative relationship between deprivation duration and overall brain size.

3.4.2 Factors underlying global alterations in brain volume

There are several potential causes that might be underlying the alterations observed in total brain volume.

First, our findings suggest that smaller total brain volumes represent a distinct neurodevelopmental effect that was independent of and disproportionate to overall growth stunting as indicated by body height and head circumference in our sample.

Second, while there are differences on a population level between Romanian individuals and individuals from other European countries in head size more generally (Chirita-Emandi, Doros, Simina, Gafencu, & Maria, 2015), these show much smaller effect sizes than the TBV effects seen in our study and cannot explain the linear relationship with deprivation duration within Romanian adoptees.

Third, another explanation would be that individuals exposed to extended, compared to limited or no deprivation, are at increased risk of having small brains for another reason – perhaps linked to exposure to genetic or pre-natal risk factors.
This was not supported by the available data on birth weight (a proxy for intrauterine exposure) or genetic risk for smaller cranial size (based on polygenic risk scores for intracranial volume). It is also theoretically unlikely given that the duration of deprivation was largely determined by the age of adoption, which in this case was linked partly to historical events (the fall of the communist regime in Romania) and partly to a range of factors such as non-standard placement practices of institutions and selection preferences of parents. Together, this strongly suggests that deprivation exposure plays a causal role in restricting brain growth.

The Romanian adoptees experienced global deprivation across a range of domains - they often lacked sufficient food and adequate hygiene, had little cognitive or social stimulation, and had no opportunity to form selective attachments with caregivers (Rutter, 1998). However, subnutrition does not appear to have played a role in the profound and long-lasting effects on brain size observed – total brain volume was largely unrelated to weight at time of adoption. We cannot rule out the possibility that the composition of institutional diets was important as this was not measured in ERA. Taken together, these findings seem consistent with the idea that psychosocial, rather than material deprivation, was responsible for the brain alterations observed. Irrespective of the specific causes of the global brain volume changes observed here, the data strongly support the idea that there is a powerful neurobiological programming effect of early exposure (Rutter & O'Connor, 2004; Weaver et al., 2004).

3.4.3 Regional alterations in brain structure

Over and above total brain volume, there were subtler regional variations in the institutionally deprived group affecting a small number of brain regions. These might be of potential relevance for cognitive and behavioural processes associated with
exposure to institutional deprivation. Institutional deprivation was associated with
greater surface area, volume and thickness in an extended region of the right
inferior temporal cortex and parahippocampus, and smaller surface area and
volume in the right inferior frontal cortex. Interestingly, surface area and volume of a
cluster in the right medial prefrontal cortex, which includes the superior frontal,
medial orbitofrontal and anterior cingulate cortices, increased as a function of
depression duration. These regions have been implicated in a broad range of
cognitive functions, from memory (parahippocampus) (Aminoff, Kveraga, & Bar,
2013) and complex visual processing (inferior temporal cortex) (Conway, 2018) to
higher-order cognitive functions like inhibition (inferior frontal cortex) (Hampshire,
Chamberlain, Monti, Duncan, & Owen, 2010), reward-guided decision making
(medial orbitofrontal cortex) (Kringelbach, 2005) and social cognition (anterior
cingulate cortex) (Fan, Duncan, de Greck, & Northoff, 2011). Impairments in all of
these functions have been reported following institutional deprivation (Golm et al.,
under submission; Kumsta, Sonuga-Barke, & Rutter, 2012; Sheridan et al., 2018;
Tibu et al., 2016).

Structural alterations in anterior cingulate and medial prefrontal cortices belong
to the most widely reported neuroimaging findings following early maltreatment
(Cohen et al., 2006; Dannlowski et al., 2012; Gold et al., 2016; Hanson et al., 2012;
Morey et al., 2015; Sheffield et al., 2013; Teicher & Samson, 2016; Thomaes et al.,
2010; Tomoda, Suzuki, et al., 2009). However, volumes are generally found to be
smaller, while our study found a positive association between deprivation duration
and surface area and volume of anterior cingulate and medial prefrontal cortices. It
should be noted that these increases in volume were relative to the global reduction
in total brain volume. It might therefore be more instructive to say that anterior
cingulate and medial prefrontal cortices were relatively less affected by deprivation
Impact of early deprivation on adult brain structure
duration compared to the rest of the cortex. Previous maltreatment studies also reported smaller volumes in dorsolateral and ventrolateral prefrontal cortex (Gold et al., 2016; Morandotti et al., 2013), including the inferior frontal cortex. In accordance with these previous findings, our study found disproportionately smaller surface area and volume associated with institutionalisation in a cluster including the right inferior frontal cortex. Inferior temporal cortex and parahippocampus have drawn less attention in maltreatment research, even though reductions in thickness and volume have been reported (Gold et al., 2016; Hanson et al., 2010; Lim et al., 2014), which seemingly stand in contrast to our finding of relatively greater thickness, surface area and volume in this region. In conclusion, while deprivation-related regional changes were located in areas that have previously been linked to early maltreatment, the positive direction of the effect in right anterior cingulate, medial prefrontal and inferior temporal cortices was surprising and is difficult to interpret.

Four points should be considered: First, the discrepancy between our findings and previous literature has multiple potential explanations such as familial confounding in previous studies or specificity of early deprivation effects in contrast to other forms of maltreatment such as abuse. Second, all brain structural alterations observed here were relative and disproportionate to the global effect of TBV loss. Clusters in inferior temporal and medial prefrontal cortices were relatively bigger compared to TBV, which might indicate that they were relatively less affected, while the cluster in inferior frontal cortex was disproportionately affected by volume loss relative to TBV. Second, not all brain alterations following childhood maltreatment necessarily represent impairment, but might also represent compensatory changes or resilience markers, as has been hypothesised by Teicher et al. (2016). Third, greater regional cortical volume should not necessarily be
regarded as being associated with better neurodevelopmental outcomes. Synaptic pruning and associated cortical volume loss are an essential part of brain development and a lack thereof can lead to impairment and disease (Tang et al., 2014). Together, the latter two points stress the importance of considering brain structural alterations in context of their link to behavioural and cognitive outcomes to identify whether they represent compensatory reorganisation of the cortex or impairment. This will be the focus of the next chapter.

Why are these brain areas particularly sensitive to early institutional deprivation? One potential reason could be their particularly rapid development during the first two years of life. Surface area of the regions observed here increases more strongly compared to the rest of the cortex: while it increases by 114% overall, surface area of right anterior cingulate cortex, medial prefrontal cortex, inferior frontal cortex (pars triangularis) and inferior temporal cortex increases between 117% - 132% (Lyall et al., 2015). However, other brain areas such as superior parietal cortex develop even more rapidly in the first two years of life and did not appear to be particularly sensitive to early deprivation in this study. Hence, early growth rates are unlikely to be the only factors indexing vulnerability to early life stress. In later development, cortical thickness starts to decrease from the age of 2 years onwards as result of synaptic pruning while surface area continues to increase until the age of 11 to 15 years before it starts to gradually decline (Gilmore et al., 2018). As the brain imaging part of this study was cross-sectional, it was not possible to identify whether relatively greater surface area and volume of the anterior cingulate, medial prefrontal and inferior temporal cortices are the result of stronger growth of these regions in early childhood development or reduced volume loss in late childhood and adolescent development (or a combination of both). Likewise, smaller surface
area and volume of the inferior frontal cortex might be the result of reduced early
growth or increased later volume loss.

3.4.4 Subcortical alterations

It was notable that there were no effects of deprivation on the volume of subcortical
structures such as the amygdala and hippocampus that were disproportionate to
TBV loss. There are a number of explanations for the disparity between this and
prior findings. First, effects of deprivation on limbic structures may be time-limited
with effects dissipating as individuals grow to adulthood. Second, studies
investigating maltreatment more generally may have failed to account for
background genetic risk that could drive both the risk exposure and the limbic
system alterations. It is also possible that different forms of maltreatment (neglect
versus abuse) may have distinct neurobiological effects. Studies of institutionally-
deprived samples may have failed to adequately control for co-occurring
psychopathology. Likewise, subcortical volumes might predict neurodevelopmental
outcomes associated with institutional deprivation in our sample, which will be
investigated in the next chapter.

3.4.5 Strengths and limitations

This study had several strengths, many of which will be discussed in more detail in
7.6. First, this was the first study to investigate the effects of early childhood
deprivation on adult brain structure. Second, the unbiased and comprehensive
whole brain approach allowed us to identify brain regions that might have been
overlooked in previous maltreatment research such as the inferior temporal cortex.
Third, the natural experimental design of this study combined with the availability of
comprehensive measures such as polygenic scores, birth weight and subnutrition
strengthened causal inferences and provided some of the most compelling evidence in the field on the long-lasting brain structural impact of early deprivation.

However, this study also had limitations. First, because of the limited sample size we were only sufficiently powered to detect medium to large effects. Second, while ERA is longitudinal, this brain imaging study was cross-sectional. It is therefore, not possible to make inferences on altered developmental trajectories of brain regions or identify brain structural effects that were time-limited to childhood or adolescence. Third, because most Romanian adoptees were placed into institutions immediately after birth, it was not possible to differentiate the effects of deprivation duration from the timing of the start of deprivation exposure. We are therefore limited in identifying sensitive periods of brain development other than highlighting the importance of the first few years. Limitations will be discussed more in 7.7.

3.4.6 Conclusion

In conclusion, early time-limited institutional deprivation is associated with substantially smaller overall brain volume in young adulthood – an effect that seems largely independent of other effects of deprivation on general physical growth. This effect did not appear to be explained by subnutrition experienced in the institutions and seems unrelated to deprivation-independent genetic and prenatal environmental risks. Beyond this global effect, there were regional alterations with relatively smaller surface area and volume in the right inferior frontal cortex, relatively bigger surface area thickness and volume in the right inferior temporal cortex. Deprivation duration was associated with relatively greater surface area and volume in the right medial prefrontal and anterior cingulate cortex. Subcortical volumes, such as amygdala and hippocampus seem largely unaffected by early institutional deprivation by young adulthood. Together, these findings provide
evidence that early adverse experiences such as institutional deprivation can long-lasting neurobiological programming effects on brain development, with changes in brain structure present more than 20 years after exposure has ended. The next chapter will explore whether these global and regional brain alterations can be linked to compensation or impairment in terms of their link to neurodevelopmental outcomes.
CHAPTER 4

DEPRIVATION-RELATED BRAIN CHANGES – MANIFEST DISORDER RISK, LATENT COGNITIVE VULNERABILITY OR NEURAL COMPENSATION
ABSTRACT

Early maltreatment has been associated with alterations in brain structure in prior studies, yet few have been able to establish a link to the forms of psychopathology often experienced by maltreated individuals. In this chapter we aimed to examine the associations between the brain structural changes caused by early childhood institutional deprivation described in the previous chapter and neurodevelopmental disorders and neuropsychological functions; to consider whether the brain changes involved mark (a) manifest disorder risk, (b) latent cognitive vulnerability (associated with cognitive deficits but not the disorder itself) or (c) neural compensation promoting resilience.

Smaller total brain volumes following deprivation predicted higher symptoms of disinhibited social engagement and lower IQ. However, the disproportionate reductions in right inferior frontal volume and relative sparing of inferior temporal volume in the institutionalised groups were associated with better neuropsychological performance and fewer symptoms of neurodevelopmental disorders. This suggests they were compensatory promoting resilience. In contrast, deprivation-related reductions in medial prefrontal cortex and anterior cingulate cortex were related to impaired empathic accuracy (but not symptoms of neurodevelopmental disorders), potentially indicating latent vulnerability.
Deprivation-related brain changes – disorder risk, vulnerability or compensation

4.1 INTRODUCTION

Early maltreatment is associated with severe and persisting negative effects on biopsychosocial development (Gilbert et al., 2009). The causal link between childhood maltreatment and later neurodevelopmental problems has been demonstrated in longitudinal prospective studies with natural experimental designs, such as the English and Romanian Adoptees (ERA) Study (Rutter, 1998). The ERA study followed the development of individuals who experienced profound global deprivation before being adopted into families that provided mostly supportive and nurturing environments. This study design strengthens causal inferences because any genetic risk for psychopathology was unrelated to both the pre-adoptive institutional and adoptive familial environment (Rutter et al., 2012). In ERA, adoption was associated with substantial and rapid recovery from the initial profound deprivation-related effects (Rutter, 1998). By age 6, the group of Romanian adoptees who spent less than 6 months in an institution was mostly indistinguishable from the group of UK adoptees, a comparison group of adoptees, who did not experience profound institutional deprivation but were adopted within the UK within the first 6 months after birth (Rutter & O'Connor, 2004). Throughout all follow-up assessments up until young adulthood these two groups showed similar neurodevelopmental outcomes (Sonuga-Barke et al., 2017). In contrast, despite early improvements, many individuals who were institutionalised for more than 6 months showed elevated symptom rates across four main neurodevelopmental domains: attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), disinhibited social engagement (DSE) and cognitive impairment (as indicated by low IQ) (Kreppner et al., 2010; Kumsta, Kreppner, et al., 2010). While the number of individuals showing increased symptoms of ADHD, ASD and DSE was persistently higher in Romanian adoptees...
Deprivation-related brain changes – disorder risk, vulnerability or compensation

with extended deprivation (>6 months), cognitive impairment (defined as IQ < 80) showed recovery and was similar to UK adoptees by the time of young adulthood (Kennedy et al., 2016; Sonuga-Barke et al., 2017). However, it should be noted that there was substantial individual variability in these outcomes. Around 50% of Romanian adoptees with extended deprivation were problem-free (with no elevated symptoms in any of the domains above) by young adulthood while 80% of adoptees who had experienced no or less than 6 months deprivation were problem-free (Sonuga-Barke et al., 2017).

In the previous chapter, we reported on the relationship between institutional deprivation and global and regional alterations in adult brain structure. Institutionalisation was associated with substantially lower total brain volumes as well as a number of regional effects once total brain volume was taken account of: lower surface area and volume in the right inferior frontal cortex and greater surface area, thickness and volume in the right inferior temporal cortex. Deprivation duration was negatively associated with total brain volume and positively with cortical surface area and volumes of the right medial prefrontal cortex and anterior cingulate cortex. This chapter sets out to answer two research questions to investigate whether brain structure is linked to deprivation-related neurodevelopmental outcomes.

5) Are deprivation-related alterations in brain structure associated with symptoms of neurodevelopmental disorders and/or neuropsychological functioning?

6) Are deprivation-related brain effects markers of manifest risk for disorder, latent cognitive vulnerability for disorder or some form of compensatory process promoting resilience?

We distinguish between three different types of brain-cognition-behaviour relations in pathways from exposure to disorder. First, neural alterations related to
Deprivation-related brain changes – disorder risk, vulnerability or compensation
deprivation may mark pathways to manifest disorder (Perry, 2010). An association
between deprivation-related brain alterations and negative outcomes (greater
disorder liability or higher levels of symptoms) would be consistent with such a
model. In fact, neurodevelopmental models typically assume the brain mediates the
relationship between maltreatment and subsequent psychopathology (Perry, 2010).
This is supported by studies that show differences between maltreated individuals
with and without a diagnosis of a psychiatric disorder. For example, smaller right
ventromedial prefrontal cortex volume (Morey et al., 2015) and smaller superior
occipital and parietal volumes (De Bellis et al., 2015) were reported for maltreated
youth with a diagnosis of posttraumatic stress disorder compared to those without
disorders. In a sample of children who experienced early institutional deprivation,
reductions in cortical thickness in frontal, parietal and temporal regions mediated
symptoms of inattention and impulsivity (McLaughlin, Sheridan, Winter, et al.,
2014).

However, most studies that have identified early maltreatment effects on the
brain do not find them related to psychopathology or disorder (for a review of these
studies, see Teicher et al., 2016). This has led researchers to raise the possibility
that brain alterations following maltreatment represent either; (1) a marker of latent
vulnerability that might not increase risk for disorders directly but would be
associated with neurocognitive deficits known to be linked to disorder - perhaps
because they were adaptive in early adverse environments but now confer long-
term vulnerability for psychopathology in normative environments such as adoptive
homes (McCrory & Viding, 2015) or (2) compensatory changes promoting resilience
rather than increased disorder risk (Teicher et al., 2016).

We could start to explore the idea of latent cognitive vulnerability because we
included a battery of neuropsychological assessments, measuring a range of
Deprivation-related brain changes – disorder risk, vulnerability or compensation processes which had been shown to be related to both (i) early deprivation or more general adversity and (ii) the neurodevelopmental disorders previously found in the ERA study. Out of this test battery, we chose three neuropsychological functions for further analysis that have previously been linked to the deprivation-related brain regions identified in Chapter 3. We predicted that (i) right inferior temporal volume would be associated with prospective memory (Aminoff et al., 2013; Cona, Scarpazza, Sartori, Moscovitch, & Bisiacchi, 2015), (ii) right inferior frontal volume would be related to inhibitory control (Meyer & Bucci, 2016; van Belle, Vink, Durston, & Zandbelt, 2014) and (iii) medial prefrontal and anterior cingulate volume would be linked to empathic accuracy (Fan, Duncan, et al., 2011). We used these neuropsychological assessments to examine the possibility that deprivation-related changes in brain structure were associated with neurocognitive alterations - even if not associated with the neurodevelopmental symptoms themselves. The concept of latent cognitive vulnerability further assumes that changes in neurocognitive functioning are predictive of future disorder risk. While we could not test this hypothesis here, these analyses provide the platform for future follow-ups of ERA to address this question directly.

Next, we explored the possibility of neural compensation occurring either during or subsequent to the exposure in a way that promotes resilience. An association between deprivation-related brain alterations and better outcomes - less symptoms would be consistent with this. As with vulnerability, resilience may be latent - associated with underlying markers of potential positive effects in neurocognitive functions but not reduced symptoms.

Overall, we hypothesized that deprivation would have heterogeneous effects on the brain manifesting as markers of manifest disorder risk, latent cognitive vulnerability to disorder and neural compensation linked to either manifest or latent
Deprivation-related brain changes – disorder risk, vulnerability or compensation resilience. Our strongest prediction was that deprivation-related reductions in total brain volume would be related to negative neurodevelopmental outcomes (increased symptoms of ADHD, ASD and DSE and low IQ) as a marker of manifest deprivation-related risk. While we examined neurodevelopmental outcomes on a symptom- rather than diagnosis-level in this study, previous reports of this sample have shown that in young adulthood, more than 29% of individuals with extended deprivation met DSM-5 diagnostic criteria for ADHD. This indicates that participants with higher symptoms in this study were likely to be at higher disorder risk. We further predicted that brain regions that were disproportionately preserved relative to the global deprivation-related reductions (right inferior temporal volume, right medial prefrontal volume) would be associated with less symptoms and better neuropsychological performance, indicating compensatory changes that promote resilience. For the right inferior frontal cortex, which showed disproportionately strong reductions following early institutional deprivation, we predicted that volume would be negatively associated with neurodevelopmental symptoms and neurocognitive functioning, indicating manifest disorder risk or latent cognitive vulnerability.
4.2 METHODS

4.2.1 Participants

The same participants as in the previous chapter were included in these analyses (see also 2.3 and 2.5.1) comprising 67 Romanian adoptees (Rom; 40.6% of the original sample, 50.7% female, mean age = 25.3 years, SD age = 1.1 years,) and 21 UK adoptees (UK, 40% of the original sample, 38.1% female, mean age = 24.4 years, SD age = 1.0 years).

4.2.2 Measures

Measures are described in detail in 2.4. The following measures were included in this chapter.

4.2.2.1 Deprivation-specific neurodevelopmental domains

To investigate the relationship between changes in brain structure and neurodevelopmental outcomes, this chapter focused on the four core neurodevelopmental problems previously associated with deprivation (Sonuga-Barke et al., 2017). These were ADHD, ASD and DSE symptoms and low IQ (as an indicator of cognitive impairment). All symptom counts were based on parent ratings obtained at ERA young adult follow-up, while IQ was assessed as part of the ERA Brain Imaging Study (ERABIS, for more detail see 2.4).

4.2.2.2 Neuropsychological assessment

In addition to the four main neurodevelopmental domains, three neuropsychological tasks were used to characterize deprivation-related alterations in behaviour and their possible relationship with brain structure changes. Prospective memory was assessed with the Memory for Intentions Test (MIST; Woods et al., 2008), proactive
Deprivation-related brain changes – disorder risk, vulnerability or compensation

inhibition with a modified Go-NoGo task (Criaud et al., 2012) and empathy with the empathic accuracy task (Mackes et al., 2018; for more detail see 3.4.2). These tasks were part of a wider battery assessing neuropsychological performance administered as part of ERABIS that were hypothesised to be sensitive to institutional deprivation. As described above - the choice to include these three tasks in this chapter was based on previous research: Each of the neuropsychological functions has been related to a brain region that we found to be sensitive to deprivation in the previous chapter.

4.2.2.3 Procedure, MRI data acquisition and processing

Please refer to 2.5 and 2.6 for a detailed description. As outlined in the previous chapter, a structural T1 scan was acquired on a 3.0 Tesla MR scanner and analysed with FreeSurfer 6.0.0.

4.2.3 Statistical analysis

4.2.3.1 Description and model selection of neurodevelopmental and -psychological behaviour

All analyses were performed in Matlab 2017a (MathWorks, 1996) and R 3.5.0 (R Core Team, 2018). To investigate the link between deprivation-related changes in brain structure and neurodevelopmental and -psychological behaviour, the distribution of these outcomes was described first in order to identify the most appropriate statistical models to use. In the case of over-dispersed count data (as is often seen in symptom counts), negative binomial regression models were chosen (‘glm.nb’ function as part of the ‘MASS’ package; Venables & Ripley, 2002). If data were non-integer interval-scaled but outcomes failed Shapiro-Wilk’s normality test because of outliers, a robust regression model was used (‘lmrob’ function as part of the ‘robustbase’ package; Maechler et al., 2018). If data followed a normal
distribution, general linear models were used unless a Breusch-Pagan test indicated heteroscedasticity (non-normal distribution of residuals). To further characterise outcomes, we tested if there were group differences between adoptees who had experienced no or only limited levels of deprivation (LoDep, combined UK and Rom who were institutionalised for less than 6 months) and adoptees who had been institutionalised for more than 6 months (Rom>6, see 2.7.2). This cut-off was chosen as previous reports on this sample have consistently shown that there was a step wise increase in neurodevelopmental problems after 6 months of deprivation while UK and Rom with less than 6 months deprivation duration did not differ (Sonuga-Barke et al., 2017).

4.2.3.2 Neurodevelopmental and neuropsychological correlates of deprivation-related global and local changes in brain structure

Total brain volume
The TBV analyses included main effects of TBV and deprivation status (LoDep vs Rom>6) and their interaction on deprivation-specific neurodevelopmental outcomes (ADHD, ASD and IQ; controlling for sex as a covariate). Our hypothesis was that a deprivation-specific effect of total brain volume would be indicated by a significant interaction term. However, if including the interaction term did not improve model fit, the main effect-only (TBV and deprivation status) model was examined. In the case of DSE symptoms, we only tested the main effect-only model within Rom>6, as there was only one individual who reported symptoms in the LoDep group.

Regional alterations cortical volume
All significant clusters previously identified as sensitive to institutionalisation or deprivation duration in the previous chapter were selected as regions of interest (ROI) and the averages of vertex-wise volume of each cluster were extracted for every participant. Only volume of each cluster was entered into the models (and not
Deprivation-related brain changes – disorder risk, vulnerability or compensation

thickness, surface area or local gyrification) to keep the number of comparisons and multicollinearity between measures low.

The three ROIs were located in the: (i) right inferior frontal cortex, (ii) right inferior temporal cortex stretching into parahippocampus, and (iii) right medial prefrontal cortex including medial orbitofrontal cortex and anterior cingulate cortex.

In the ROI analyses, the same interaction models as above were tested to see if deprivation status moderated the relationship between ROI volumes and neurodevelopmental problems (controlling for TBV and sex as covariates). All ROI volumes and their interaction terms with deprivation status were entered into one model for each neurodevelopmental problem. If the interaction model fitted significantly better compared to the main effect-only model (ROI volumes and deprivation status), the individual interaction parameters for each ROI were investigated. If including the interaction terms did not improve model fit, the main effect-only model was examined. Again, for DSE symptoms the main effect-only model within Rom>6 was tested instead.

In addition to neurodevelopmental problems, interaction analyses were also performed for each ROI separately with a neuropsychological outcome as dependent variable that we hypothesised to be related to that ROI (i.e. prospective memory, proactive inhibition or empathic accuracy).

Subcortical volumes

In the subcortical analyses, the averages of left and right volumes of each subcortical structure were entered as predictors into one model. First, the interaction model between deprivation status and subcortical volumes on neurodevelopmental problems was tested (controlling for TBV and sex as covariates). If the interaction model had a significantly better fit compared to the main effect-only model (subcortical volumes and deprivation status), the individual
Deprivation-related brain changes – disorder risk, vulnerability or compensation

interaction terms were investigated, otherwise the main effect-only model was tested. As in the other analyses, for DSE symptoms only the main effect-only model within Rom>6 was investigated.
4.3 RESULTS

4.3.1 Distribution and model selection

Distributions and descriptive statistics of the main deprivation-specific neurodevelopmental domains (ADHD, ASD and DSE symptoms and IQ) and additional neuropsychological measures (prospective memory, proactive inhibition and empathic accuracy) are shown in Figure 4.1 and Table 4.1. All symptom counts were over-dispersed, with variance far exceeding the mean for each group. Negative binomial models and non-parametric Spearman correlation coefficients were therefore used for these symptoms. IQ, on the other hand, was normally distributed (Shapiro-Wilk (SW)=0.98, p=0.37) and linear regressions were applied in subsequent analyses (Figure 4.1).

Proactive inhibition was normally distributed and linear regressions were used (SW=0.97, p=0.53), but prospective memory and empathic accuracy were non-normally distributed due to outliers (prospective memory: SW=0.82, p<0.001, empathic accuracy: SW=0.95, p<0.01). Moreover, prospective memory had a ceiling effect with 18 out of 87 participants achieving maximum scores. Consequently, for further analyses robust regressions (see 4.2.3) were used.
4.3.2 Neurodevelopmental correlates of deprivation

Compared to LoDep, Rom>6 had significantly higher rates of ADHD and DSE symptoms, lower IQ and marginally higher rates of ASD symptoms (ADHD: Likelihood Ratio $(LR) \chi^2(1, 78)=6.82, p<0.01$; ASD: $LR \chi^2(1, 75)=3.68, p=0.06$; DSE: $LR \chi^2(1, 76)=17.89, p<0.001$; IQ: $F(1, 86)=15.71, p<0.001$, Table 4.1). There were no significant differences between these groups in prospective memory.
Deprivation-related brain changes – disorder risk, vulnerability or compensation

\( (\text{Deviance } \chi^2(1,85)=0.10, p=0.75) \) and proactive inhibition \( (F(1,80)=2.80, p=0.10) \), while empathic accuracy scores were lower in Rom>6 \( (\text{Deviance } \chi^2(1,81)=3.86, p<0.05) \).

Table 4.1: Descriptive statistics of neurodevelopmental domains by deprivation status (LoDep vs Rom>6). Significant group differences are marked in bold.

<table>
<thead>
<tr>
<th>Neurodevelopmental domain</th>
<th>group</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
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<tbody>
<tr>
<td>ADHD symptoms</td>
<td>LoDep</td>
<td>40</td>
<td>1.98</td>
<td>3.32</td>
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<td>15</td>
<td>( LR \chi^2=6.82, p&lt;0.01 )</td>
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<tr>
<td></td>
<td>Rom&gt;6</td>
<td>40</td>
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<td>0.00</td>
<td>14</td>
<td>( LR \chi^2=3.68, p=0.06 )</td>
</tr>
<tr>
<td></td>
<td>Rom&gt;6</td>
<td>39</td>
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</tr>
<tr>
<td>DSE symptoms</td>
<td>LoDep</td>
<td>39</td>
<td>0.05</td>
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<td>0.00</td>
<td>2</td>
<td>( LR \chi^2=17.89, p&lt;0.001 )</td>
</tr>
<tr>
<td></td>
<td>Rom&gt;6</td>
<td>39</td>
<td>0.77</td>
<td>1.04</td>
<td>0.00</td>
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<td></td>
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<td>IQ</td>
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<td>Rom&gt;6</td>
<td>46</td>
<td>92.35</td>
<td>11.75</td>
<td>93.50</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>prospective memory</td>
<td>LoDep</td>
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<td>41.71</td>
<td>6.28</td>
<td>43.50</td>
<td>27.00</td>
<td>( \text{Deviance } \chi^2=0.10, p=0.75 )</td>
</tr>
<tr>
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<td>Rom&gt;6</td>
<td>45</td>
<td>40.38</td>
<td>7.88</td>
<td>42.00</td>
<td>37.00</td>
<td></td>
</tr>
<tr>
<td>proactive inhibition</td>
<td>LoDep</td>
<td>41</td>
<td>33.85</td>
<td>27.88</td>
<td>33.46</td>
<td>115.59</td>
<td>( F=2.80, p=0.10 )</td>
</tr>
<tr>
<td></td>
<td>Rom&gt;6</td>
<td>41</td>
<td>23.58</td>
<td>27.68</td>
<td>21.64</td>
<td>136.14</td>
<td></td>
</tr>
<tr>
<td>empathic accuracy</td>
<td>LoDep</td>
<td>40</td>
<td>1.05</td>
<td>0.18</td>
<td>1.06</td>
<td>1.03</td>
<td>( \text{Deviance } \chi^2=3.86, p&lt;0.05 )</td>
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<tr>
<td></td>
<td>Rom&gt;6</td>
<td>43</td>
<td>0.97</td>
<td>0.21</td>
<td>0.99</td>
<td>0.92</td>
<td></td>
</tr>
</tbody>
</table>

LoDep – low deprivation group; Rom>6 – Romanian adoptees, who were institutionalised for more than 6 months; n – sample size; SD – standard deviation. LR \( \chi^2 \) – Likelihood Ratio Test; Deviance \( \chi^2 \) – Robust Deviance Test.

4.3.3 Relationship between outcomes

Across both groups, all deprivation-specific neurodevelopmental problems were significantly correlated (all \( p<0.05 \); Figure 4.2a). Beyond this, ADHD and ASD symptoms were significantly negatively correlated with prospective memory, proactive inhibition and empathic accuracy (all \( p<0.05 \); Figure 4.2a). DSE symptoms were negatively related to empathic accuracy \( (p<0.001) \) and IQ was
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positively correlated with prospective memory ($p<0.001$) and empathic accuracy ($p<0.01$).

**Figure 4.2: Correlation between behavioural outcomes.** Spearman correlations were performed. Colours indicate the strength and direction of the correlation coefficient (negative – blue, positive -red). Asterisks indicate significance levels: * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$.

### 4.3.4 Neurodevelopmental correlates of total brain volume

There was no interaction between TBV and deprivation status on ADHD ($p=0.59$) or ASD symptoms ($p=0.22$), nor was there a main effect of TBV irrespective of deprivation status (ADHD: $p=0.12$; ASD: $p=0.73$). DSE symptoms was negatively associated with TBV in Rom>6 ($LR \chi^2=(1,36)= 4.91, p<0.05$, Figure 4.3a). On average, a decrease of 161 cm$^3$ in TBV was associated with an increase of 1 symptom score. There was no interaction between deprivation status and TBV on IQ ($p=0.71$). However, TBV negatively predicted IQ independently of deprivation status, with an average increase of 5 IQ points for every additional 100 cm$^3$ in TBV ($B=0.05, F(1,84)=7.56, p<0.01$, Figure 4.3b).
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Figure 4.3: Neurodevelopmental correlates of total brain volume. a) In Rom>6, total brain volume negatively predicted DSE symptoms. b) Irrespective of deprivation status, total brain volume positively predicted IQ. Shaded areas depict 95% confidence intervals around the regression lines. Regressions included sex as a covariate of no interest.

4.3.5 Neurodevelopmental and -psychological correlates of regional brain alterations

Next, we explored the relationship between neurodevelopmental outcomes and regional alterations of the brain regions identified in Chapter 3 as sensitive to institutionalisation (clusters in the right inferior frontal gyrus and right inferior temporal cortex) and deprivation duration more specifically (cluster in the right medial prefrontal cortex).

Deprivation status moderated the relationship between ROI volumes and ADHD symptoms ($LR \chi^2(3,70)=10.16, p<0.05$), with significant interaction terms for right inferior temporal volume ($LR \chi^2(1,70)=4.80, p<0.05$) and anterior cingulate volume ($LR \chi^2(1,70)=7.92, p<0.01$). Post-hoc tests revealed that in Rom>6, there was a significant negative relationship between right inferior temporal volume and ADHD symptoms, whereas inferior temporal volume was positively, albeit non-significantly, related to ADHD symptoms in the LoDep group (Rom>6: $B=-2.19, p<0.001$; LoDep: $B=0.72, p=0.40$; Figure 4.4). In contrast, the relationship between right anterior cingulate volume and ADHD symptoms was significant and negative in the LoDep
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group \((B=-2.62, p<0.01)\), while this association was non-significant in Rom>6
\((B=1.35, p=0.89)\).

There were no significant interactions between deprivation status and the ROIs
on ASD symptoms \((p=0.33)\) or IQ \((p=0.55)\) nor were there significant main effects of
ROIs across groups (ASD: \(p=0.08\), IQ: \(p=0.08\)). There was also no significant main
effect of these brain regions on DSE symptoms in Rom>6 \((p=0.47)\).

There was a significant interaction between deprivation status and right inferior
frontal volume on proactive inhibition \((F(1,76)=4.61, p<0.05)\). In Rom>6 this
relationship was negative while it was non-significant and positive in LoDep
(Rom>6: \(B=-31.69, p<0.05\), LoDep: \(B=10.37, p=0.42\)).

Deprivation status moderated the association between right inferior temporal
volume and prospective memory \((Deviance X^2(1,82)=3.93, p<0.05)\), with a non-
significant positive relationship Rom>6 and a non-significant positive association in
LoDep (Rom>6: \(B=2.28, p=0.88\); LoDep: \(B=-3.59, p=0.10\)).

Finally, in Rom>6, anterior cingulate volume negatively predicted empathic
accuracy while this association was positive in LoDep (Rom>6: \(B=-0.22, p<0.05\);
LoDep: \(B=0.17, p<0.05\); interaction: \(Deviance X^2(1,78)=8.19, p<0.01\)).

In both the inferior temporal and inferior frontal cortex, greater volumetric
differences compared to the UK group were associated with better
neuropsychological performance or lower ADHD symptoms for Romanian adoptees
with extended deprivation exposure. In the case of inferior temporal volume,
institutionalisation was associated with greater volumes, which in turn predicted less
ADHD symptoms and better prospective memory in Rom>6. For inferior frontal
volume, smaller volumes following institutionalisation were associated with better
proactive inhibition in the highly deprived group. We interpret this as preliminary
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evidence that these local alterations partly reflect compensatory adaptations in cortical structure.

In contrast, deprivation-duration-related increases in anterior cingulate volume were associated with worse outcomes in Rom>6. First, while greater anterior cingulate volumes predicted lower ADHD symptoms in the LoDep group, this apparently beneficial effect disappeared in Rom>6. Moreover, greater anterior cingulate volumes were associated with lower empathic accuracy, a relationship reversed compared to the one seen in LoDep. This might indicate that deprivation-duration-related changes in anterior cingulate volume reflect latent cognitive vulnerability.
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Figure 4.4: Interactions between regional brain volumes and deprivation status on neurodevelopmental and neuropsychological performance. Regions of interest are highlighted in blue on the brain maps. (i) Right inferior frontal volumes were more negatively associated with proactive inhibition in Rom>6 compared to LoDep. (ii) Right inferior temporal volumes were more negatively associated with ADHD symptoms and more positively with prospective memory in Rom>6. (iii) Anterior cingulate volumes were more positively associated with ADHD symptoms and more negatively with empathic accuracy in Rom>6. Shaded areas depict 95% confidence intervals around the regression lines. Asterisks indicate significance of the individual group slopes with * - p<0.05, ** - p<0.01, *** - p<0.001.

4.3.6 Neurodevelopmental correlates of subcortical volumes

There was no significant interaction between deprivation status and subcortical volumes for any neurodevelopmental outcome (ADHD symptoms: p=0.07; ASD symptoms: p=0.29; IQ: p=0.49), nor were there significant main effects of subcortical volumes on these outcomes (ADHD symptoms: p=0.05; ASD symptoms: p=0.27; DSE symptoms p=0.31; IQ: p=0.54).
4.4 DISCUSSION

Brain plasticity is a double-edged sword and while it leaves individuals vulnerable to the effects of adversity (in this case institutional deprivation), it also offers the promise of recuperation, recovery and compensation. In fact, our data fit with a model whereby global and some of the local effects of institutional deprivation observed here reflect manifest disorder risk and latent cognitive vulnerability, while others are the product of compensatory cortical restructuring occurring either within the institutions or subsequently in the participants’ adoptive homes and promoting manifest and latent resilience.

4.4.1 Total brain volume

While TBV was unrelated to ADHD and ASD symptoms, it was positively associated with IQ, irrespective of and controlling for deprivation status. A moderate positive association between TBV and IQ has been documented in the general population (Deary, Penke, & Johnson, 2010; McDaniel, 2005) and it is therefore not surprising that we found this effect across groups. The relationship between TBV and IQ is thought to partly result from common underlying genetic factors (Bohlken et al., 2016). However, studies have also shown that the relationship between TBV and IQ can still be observed even if reductions in TBV are the result of environmental influences. For example, adolescents who were born extremely preterm have smaller TBV and lower IQ, with TBV explaining about 30% of the difference in IQ between the preterm-born and control groups (i.e. Cheong et al., 2013). Consistent with this, our study suggests that lower general intelligence in the extended deprivation group can partly be explained by smaller TBV volumes caused by institutional deprivation.
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DSE symptoms increased as TBV declined in the extended deprivation group. This finding is difficult to interpret as little is currently known about the underlying pathophysiology of DSE. DSE has been linked to a lack of selectivity in attachment (Kennedy et al., 2017; Lawler, Koss, Doyle, & Gunnar, 2016), which has been proposed to be an evolutionary adaptation to a lack of a preferred attachment figure as often experienced in institutional settings (Balbernie, 2010). Consequently, one possibility might be that DSE symptoms are a more specific marker of social care quality and one-on-one caregiver contact in institutions and therefore further evidence for the psychosocial aspects of deprivation as determinants for smaller TBV. Together, these findings implicate that the profound effect of institutional deprivation on TBV constitutes a marker of manifest risk for neurodevelopmental problems in young adulthood.

4.4.2 Regional alterations of cortical volume

In the group of adoptees with extended deprivation, the institutionalisation-related increase in inferior temporal volume and reduction in inferior frontal volume were associated with better performance on neuropsychological tasks tapping prospective memory and proactive inhibition, respectively, which might indicate latent resilience. Interestingly, increases in inferior temporal volume were also related to lower levels of ADHD symptoms, which might represent manifest resilience. Taken together, we interpret this as preliminary evidence that these regional effects of institutionalisation indicate compensation rather than disorder risk and are driven by individuals in the Romanian adoptee group with better neurodevelopmental outcomes. These findings are broadly in line with the hypothesis advanced by Teicher and Samson (2013) that many brain alterations
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seen following maltreatment might be adaptive (at least in adverse environments) as they often occur in the absence of psychopathology.

The deprivation duration effects seen in right anterior cingulate and medial prefrontal cortices (larger volumes with more extended deprivation), were on the other hand, related to lower empathic accuracy and did not exert the protective effects on ADHD symptoms that were observed in the low deprivation group. Especially with regard to empathic accuracy, which is defined as the ability to accurately infer others’ emotions (Ickes, Stinson, Bissonnette, & Garcia, 1990), it is striking how deprivation exposure altered the relationship between volume in this brain region and behaviour. In line with the latent cognitive vulnerability model (McCrory & Viding, 2015), this finding suggests that reduced empathic accuracy is a neurocognitive alteration associated with extended deprivation, which can partly be explained by disproportionate volume increases in the right anterior cingulate and medial prefrontal cortices. Indeed, deficits in empathic accuracy might confer an increased risk for psychopathology as they were associated with worse outcomes in all neurodevelopmental symptom domains analysed in this study and future follow-ups of this study will be able to test whether changes in empathic accuracy reported here predict future disorder risk. Relatedly, the last follow-up of the ERA study found that callous-unemotional traits, which are associated with a lack of empathy, were higher in Romanian adoptees who met the diagnostic criteria for DSE and ADHD (Kennedy et al., 2017; Kennedy et al., 2016).

4.4.3 Subcortical volumes

We found no evidence that subcortical volumes were related to neurodevelopmental outcomes or that their link to these outcomes was moderated by deprivation status. There are a couple of possible explanations for these negative findings. First,
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Volumetric differences related to neurodevelopmental outcomes might be present in childhood but disappear in later development. This is partly supported by findings of the ENIGMA consortium that found differences in subcortical structures in childhood but not in adulthood in a large dataset of patients with ADHD and healthy controls (Hoogman et al., 2017). However, compared to healthy controls, patients with ASD had significantly smaller volumes of putamen, pallidum and nucleus accumbens irrespective of age but with relatively small effect sizes (van Rooij et al., 2017).

Another possibility for our negative findings might therefore be that we did not have enough power to detect small subcortical effects on neurodevelopmental outcomes, especially when considering that symptom levels in this sample were overall lower than in a clinical sample of individuals with a diagnosis ADHD or ASD.

4.4.4 Strengths and limitations

This chapter had several strengths. First, the availability of a comprehensive set of neurodevelopmental and neuropsychological measures allowed us to systematically investigate the relationship between deprivation-related changes in brain structure and behavioural outcomes. Second, with the inclusion of a comparison group with no or limited deprivation we were able to test for interactions between deprivation status and brain structure on behaviour, providing first evidence for altered brain-behaviour-links following extended deprivation.

This chapter also had limitations. Neurodevelopmental problems were investigated on a symptom level. While this allowed continuous dose-dependent relationships to be identified, inferences on individuals meeting diagnostic criteria for a neurodevelopmental disorder might be limited. Moreover, this chapter focused on regions of interest and neuropsychological outcomes that had previously been functionally associated with these brain regions. It is therefore likely that we have
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not identified all brain regions associated with a certain outcome (this will be explored more in the next chapter). Vice versa, brain regions are likely to be associated with more outcomes than the ones investigated here. Furthermore, we were only able to test direct linear relationships between regions of interest and neurodevelopmental and neuropsychological outcomes. The actual pathways between early exposure, changes in brain structure and behaviour are likely to be multifaceted, iterative and reciprocal with additional direct and indirect links via social functioning and environment. Strengths and limitations of this study in general will be discussed in 7.6 and 7.7.

4.4.5 Conclusion

In conclusion, this study provides first evidence that some brain structural changes following institutional deprivation might represent manifest disorder risk and latent cognitive vulnerability while others may be related to compensation promoting manifest and latent resilience (e.g., leading to preserved proactive inhibition). Reductions in total brain volume were associated with lower general intelligence and higher DSE symptoms. In contrast, institutionalisation-related regional differences in the inferior frontal and inferior temporal gyri were associated with better outcomes and therefore may represent compensatory alterations in cortical structure. However, deprivation duration-related alterations in medial prefrontal and anterior cingulate volume were associated with impaired empathic processing which in turn might increase risk for developing disorders related to empathic deficits.
CHAPTER 5

DOES DEPRIVATION STATUS MODERATE THE RELATIONSHIP BETWEEN BRAIN STRUCTURE AND ADHD SYMPTOMS, ASD SYMPTOMS OR LOW IQ?
Deprivation moderates link between brain structure and symptoms

ABSTRACT

Deprivation-specific neurodevelopmental problems such as ADHD and ASD have a different aetiology compared to their normative, idiopathic clinical variants - with the latter thought to be more strongly genetic in nature. However, it remains unclear whether deprivation-related variants have distinct neurobiological signatures compared to non-deprivation-related variants. We provided initial evidence for this in Chapter 4 based on an region of interest (ROI) analysis strategy. In this chapter we aimed to explore this question more systematically through a whole brain analysis examining whether the association between brain structure and attention-deficit/hyperactivity disorder (ADHD) symptoms, autism spectrum disorder (ASD) symptoms or IQ differed in those exposed to no or low levels of deprivation (combining the UK and Romanian adoptees exposed to less than 6 months deprivation; LoDep) versus those exposed to extended deprivation (more than 6 months; Rom>6). Deprivation status moderated the association between brain structure and symptoms of ADHD and ASD and IQ. ADHD symptoms were associated with increased gyrification in bilateral supramarginal, right lingual and right superior frontal cortices in Rom>6 but not in LoDep. Similarly, ASD symptoms were linked to increased surface area in the left superior parietal cortex in Rom>6 but not LoDep. In Rom>6, IQ was associated with increased surface area and volume in the left lingual cortex, smaller thickness in the left caudal middle frontal cortex and smaller volume in the right superior parietal gyrus, while these relationships were non-significant or reversed in LoDep. These findings provide initial support for the notion that deprivation-specific disorders may have a distinctive neural signature. Future studies need to extend this analysis to a comparison of brain structure in deprived and normative clinical cases.
5.1 INTRODUCTION

Early childhood maltreatment is associated with increased risk for psychopathology (Gilbert et al., 2009). More so, maltreatment-related psychiatric disorders may be qualitatively different from their idiopathic variants (Teicher & Samson, 2013). This is because individuals with psychopathology and a history of childhood maltreatment may differ in several ways from those with the same diagnosis but without a history of childhood maltreatment (McCrory et al., 2017; Teicher et al., 2016). With regard to psychiatric disorders such as depression and generalised anxiety disorder, there is mounting evidence that maltreated individuals show an earlier age of onset, more severe symptoms, higher levels of comorbidity, higher suicide risk and poorer response to treatment compared to individuals with the same diagnosis but without a history of maltreatment (Teicher & Samson, 2013). In the English and Romanian Adoptees (ERA) Study, exposure to institutional deprivation for more than 6 months was associated with a specific and persistent pattern of neurodevelopmental problems (Rutter & O’Connor, 2004; Sonuga-Barke et al., 2017). Extended deprivation (>6 months) was associated with increased symptoms of ADHD with a pattern that showed certain characteristics which differed from common idiopathic variants of the disorder. Not only was ADHD very prevalent, with seven times higher risk of meeting diagnostic criteria following more than 6 months deprivation (Kennedy et al., 2016). It was also particularly persistent with little recovery up until young adulthood, manifested mainly as inattention rather than hyperactivity or impulsivity, was equally common in females and males and showed low comorbidity with conduct disorder (Kennedy et al., 2016). Individuals with extended deprivation were also more likely to show increased rates of ASD symptoms throughout development (Sonuga-Barke et al., 2017), a condition that is thought to be mostly genetically determined in the general population (Tick, Bolton,
Deprivation moderates link between brain structure and symptoms

Happé, Rutter, & Rijsdijk, 2016) but seems to have an environmental cause in ERA. Extended deprivation was also linked to higher rates of disinhibited social engagement (DSE), a condition that describes inappropriate, overly familiar behaviour towards strangers in children who experienced neglect (American Psychiatric Association, 2013) with DSE behaviour often persisting into young adulthood (Sonuga-Barke et al., 2017). Finally, individuals with extended deprivation showed initially higher levels of cognitive impairment (indicated by IQ < 80; Rutter & O’Connor, 2004), which recovered by young adulthood (Sonuga-Barke et al., 2017). This indicates the potential of neuroplastic recovery, which stands in contrast to relatively high stability of IQ from young childhood onwards in the general population (Schneider, Niklas, & Schmiedeler, 2014). Together, these findings provide evidence that – in line with the hypothesis advanced by Teicher and Samson (2013) – deprivation-related variants of neurodevelopmental problems are distinct from their non-deprivation-related counterparts and might consequentially show distinct brain structural correlates.

Some studies have shown differences between brain structure of patients who have the same psychiatric diagnosis, such as schizophrenia, but differ in their history of childhood maltreatment (Opel et al., 2014; Sheffield et al., 2013; Vythilingam et al., 2002). For ERA, the previous chapter has provided some evidence that deprivation status moderated the link between institutionalisation-related brain alterations and neurodevelopmental problems: Increases in right inferior temporal volume were negatively associated with ADHD symptoms in the extended deprivation group but not the low deprivation group. This could be interpreted as intial evidence for a deprivation-related, neurobiologically distinct variant of ADHD. However, the previous chapter only investigated limited cortical and subcortical ROIs to test specific hypotheses. In this chapter we will explore this
Deprivation moderates link between brain structure and symptoms

issue further at the level of the whole cortex by performing whole-brain analyses to investigate whether deprivation status moderates the relationship between neurodevelopmental problems (symptoms of ADHD, ASD and DSE and IQ) and cortical brain structure. We will thereby continue to explore symptoms and functioning on a continuous level rather than comparing individuals who meet diagnostic criteria for the respective disorders.
5.2 METHODS

5.2.1 Participants

The same participants as in the previous chapter were included in these analyses (see also 2.3 and 2.5.1) comprising 67 Romanian adoptees (Rom; 40.6% of the original sample, 50.7% female, mean age=25.3 years, SD age=1.1 years,) and 21 UK adoptees (UK, 40% of the original sample, 38.1% female, mean age=24.4 years, SD age=1.0 years).

5.2.2 Measures

Measures used in ERABIS are described in detail in 2.4. The following measures were used in the analyses reported in this chapter.

5.2.2.1 Deprivation-specific neurodevelopmental domains

To investigate the relationship between changes in brain structure and neurodevelopmental outcomes, this chapter focused on the four core neurodevelopmental problems previously associated with deprivation (Sonuga-Barke et al., 2017). These were ADHD, ASD and DSE symptoms and IQ (as an indicator of cognitive impairment). All symptom counts were based on parent ratings obtained at ERA young adult follow-up, while IQ was assessed as part of the ERA Brain Imaging Study (ERABIS).

5.2.2.2 Procedure, MRI data acquisition and processing

Please refer to 2.5 and 2.6 for a detailed description. As outlined in the previous chapter, a structural T1 scan (acquired on a 3.0 Tesla MR scanner) was analysed with FreeSurfer 6.0.0 to quantify cortical thickness, surface area, volume and local gyrification index as well as subcortical volumes. For cortical thickness, surface
area and volume, smoothing was performed with a 10 mm kernel at full-width/half-max (FWHM), whereas a 5 mm FWHM kernel was applied for local gyrification because it is inherently smoother.

5.2.3 Statistical analysis

To examine the impact of deprivation status, we compared individuals with no or only limited deprivation exposure (UK and Romanian adoptees who were institutionalised for less than 6 months, LoDep) with individuals who experienced extended deprivation of more than 6 months (Rom>6). This cut-off is consistent with previous reports that have shown a step-wise increase in neurodevelopmental symptoms after 6 months of deprivation duration and no differences between UK and Rom who were institutionalised for less than 6 months (see 2.7).

Whole-brain analyses were performed with FreeSurfer 6.0.0 to test whether there were significant interactions between deprivation status (LoDep versus Rom>6) and neurodevelopmental outcomes (symptoms of ADHD or ASD or low IQ) on any brain regions across the whole cortex. Regional cortical volume, surface area, thickness and gyrification were all investigated. We only tested the main effect of DSE symptoms on brain structure within the Rom>6 group as there was only one individual with elevated DSE symptoms in the LoDep group.

As in the whole-brain analyses reported in Chapter 3, sex and TBV were entered as covariates of no interest in the volume, surface area and local gyrification analyses to examine whether there were effects which were not proportional to TBV. As cortical thickness does not scale closely with TBV, we only controlled for sex in those analyses (Barnes et al., 2010). Cluster-wise correction for multiple comparisons was performed using a Monte Carlo simulation (vertex-wise threshold $p<.05$, clusterwise-threshold $p<.05$).
5.3 RESULTS

As described in the previous chapter, compared to the LoDep group, Rom>6 had significantly higher rates of ADHD and DSE symptoms, lower IQ and marginally higher rates of ASD symptoms (Table 5.1).

Table 5.1: Descriptive statistics of neurodevelopmental domains by deprivation status (LoDep vs Rom>6). Significant group differences are marked in bold.

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<td>ADHD symptoms</td>
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</table>

LoDep – low deprivation group; Rom>6 – Romanian adoptees, who were institutionalised for more than 6 months; n – sample size; SD – standard deviation. LR X² – Likelihood Ratio Test; Deviance X² – Robust Deviance Test.

There were multiple clusters across the cortex which showed a significant interaction between ADHD symptoms and deprivation status on patterns of gyrification. We found a widespread pattern of increased gyrification associated with ADHD symptoms in Rom>6, but mostly non-significant associations between ADHD symptoms and gyrification in the LoDep group. Post-hoc tests revealed that gyrification indices of clusters located in (i) bilateral supramarginal, (ii) right lingual and (iii) right superior frontal cortices were positively associated with ADHD symptoms in the Rom>6 group but non-significant in the LoDep group. In contrast, a cluster in the (iv) right rostral middle frontal cortex showed a significant negative
Deprivation moderates link between brain structure and symptoms association with ADHD symptoms in LoDep but no significant association in Rom>6 (Figure 5.1, Table 5.2). No interactions between deprivation status and ADHD symptoms were found for cortical surface area, thickness or volume.

Deprivation status also moderated the relationship between ASD symptoms and regional brain structure: There was a significant interaction in surface area in a cluster including the left superior parietal cortex which showed a significant positive association with ASD symptoms in Rom>6 but a non-significant negative relationship in LoDep (Figure 5.1, Table 5.2). No clusters were identified that showed a significant interaction between deprivation status and ASD symptoms in cortical thickness, volume or gyrification.

Figure 5.1: Significant interactions between deprivation status and ADHD symptoms on local gyrification and ASD symptoms on surface area. Each symbol represents a different individual and the shaded areas represent 95% confidence intervals around the regression line. Asterisks indicate significant correlations for each subgroup, which were calculated post-hoc based on the average vertex-wise measure of each significant cluster.

Finally, deprivation status moderated the relationship between IQ and cortical structure in a number of regions including the left lingual cortex, left superior frontal cortex and right postcentral gyrus. In the left lingual cortex, correlations between IQ and surface area and volume were positive in the Rom>6 group but
Deprivation moderates link between brain structure and symptoms

non-significant in the LoDep group. IQ was negatively associated with left caudal middle frontal cortex thickness and right superior parietal gyrus volume in the Rom>6 group, while IQ was positively correlated with thickness and volume in these two regions in the LoDep group (Figure 5.2, Table 5.2).

**Figure 5.2: Significant interactions between deprivation status and IQ on local cortical structure.** Each symbol represents a different individual and the shaded areas represent 95% confidence intervals around the regression line. Asterisks indicate significant correlations within each subgroup, which were calculated post-hoc based on the average vertex-wise measure of each significant cluster.
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Table 5.2: Clusters showing significant interactions between deprivation status (LoDep versus Rom>6) and neurodevelopmental outcomes on cortical volume, surface area, thickness or local gyrification. All results were corrected for multiple comparisons using Monte Carlo correction (clusterwise-threshold \(p<0.05\)). Effect sizes \((R^2)\) for the interaction effect of each cluster were derived from whole brain effect size maps. Effect sizes \((r)\) of each group were calculated post-hoc on the basis of average vertex-wise measures for each significant cluster.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Anatomical region</th>
<th>H</th>
<th>Cluster size [mm(^2)]</th>
<th>MNI coordinates [mm]</th>
<th>Clusterwise p</th>
<th>Effect Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interactions between deprivation status and ADHD symptoms</td>
<td>local gyrification supra-marginal</td>
<td>L</td>
<td>8507</td>
<td>-54 -20 -33</td>
<td>0.0002</td>
<td>Interaction: (R^2=0.07) LoDep: (r=-0.30) Rom&gt;6: (r=0.40)</td>
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<tr>
<td></td>
<td>local gyrification supra-marginal</td>
<td>R</td>
<td>5174</td>
<td>47 -71 7</td>
<td>0.0002</td>
<td>Interaction: (R^2=0.08) LoDep: (r=-0.27) Rom&gt;6: (r=0.36)</td>
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<tr>
<td></td>
<td>local gyrification lingual</td>
<td>R</td>
<td>4197</td>
<td>12 -82 -9</td>
<td>0.0300</td>
<td>Interaction: (R^2=0.07) LoDep: (r=-0.24) Rom&gt;6: (r=0.42)</td>
</tr>
<tr>
<td></td>
<td>local gyrification superior frontal</td>
<td>R</td>
<td>2861</td>
<td>21 -16 65</td>
<td>0.0086</td>
<td>Interaction: (R^2=0.09) LoDep: (r=-0.29) Rom&gt;6: (r=0.34)</td>
</tr>
<tr>
<td></td>
<td>local gyrification rostral middle frontal</td>
<td>R</td>
<td>3508</td>
<td>36 55 -8</td>
<td>0.0018</td>
<td>Interaction: (R^2=0.07) LoDep: (r=-0.43) Rom&gt;6: (r=0.27)</td>
</tr>
</tbody>
</table>

Interaction between deprivation status and ASD symptoms

<table>
<thead>
<tr>
<th>Measure</th>
<th>Anatomical region</th>
<th>H</th>
<th>Cluster size [mm(^2)]</th>
<th>MNI coordinates [mm]</th>
<th>Clusterwise p</th>
<th>Effect Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>area superior parietal</td>
<td>L</td>
<td>2149</td>
<td>-20 -63 48</td>
<td>0.0010</td>
<td>Interaction: (R^2=0.09) LoDep: (r=-0.31) Rom&gt;6: (r=0.47)</td>
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</table>

Interactions between deprivation status and IQ

<table>
<thead>
<tr>
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<th>Anatomical region</th>
<th>H</th>
<th>Cluster size [mm(^2)]</th>
<th>MNI coordinates [mm]</th>
<th>Clusterwise p</th>
<th>Effect Sizes</th>
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</thead>
<tbody>
<tr>
<td>volume lingual</td>
<td>L</td>
<td>907</td>
<td>-11 -90 -2</td>
<td>0.0294</td>
<td>Interaction: (R^2=0.18) LoDep: (r=-0.21) Rom&gt;6: (r=0.34)</td>
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<tr>
<td>area lingual</td>
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<td>-13 -84 2</td>
<td>0.0002</td>
<td>Interaction: (R^2=0.46) LoDep: (r=-0.23) Rom&gt;6: (r=0.32)</td>
<td></td>
</tr>
<tr>
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<td>-41 15 47</td>
<td>0.0124</td>
<td>Interaction: (R^2=0.07) LoDep: (r=0.41) Rom&gt;6: (r=-0.38)</td>
<td></td>
</tr>
<tr>
<td>volume superior parietal</td>
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<td>914</td>
<td>16 -43 70</td>
<td>0.0140</td>
<td>Interaction: (R^2=0.07) LoDep: (r=0.36) Rom&gt;6: (r=0.44)</td>
<td></td>
</tr>
</tbody>
</table>

DSE symptoms in Rom>6 group

no significant clusters

H: hemisphere; L: left; R: right; \(r\): partial correlation coefficient, LoDep: low deprivation group; Rom>6: extended deprivation group who experienced more than 6 months of deprivation
5.4 Discussion

Twin studies show that neurodevelopmental disorders such as ADHD and ASD and more general cognitive functioning (IQ) are highly heritable suggesting a strong genetic element (Haworth et al., 2010; Larsson, Chang, D’Onofrio, & Lichtenstein, 2014; Tick et al., 2016). The ERA study, and other high-risk adoption studies, provide evidence that in extremis these conditions might have a largely environmental cause. The question of whether these latter variants of common disorders have the same neural signatures as non-deprivation related variants of these conditions has potentially far-reaching clinical implications, as it may determine the most appropriate treatment target and mechanism of action for deprived children suffering from or at increased risk of developing mental disorders (McCrory et al., 2017). Here we started to explore this idea by examining whether the associations between brain structure alterations and neurodevelopmental symptoms were moderated by deprivation status. We wanted to investigate whether deprivation-related symptoms or IQ deficits had distinct neuroanatomical correlates compared to neurodevelopmental symptoms or IQ reductions seen in children with limited experience of deprivation. We obtained compelling evidence that, at least with regard to some brain measures, the neural signatures of ADHD or ASD symptoms in the Rom>6 group were different from those observed in the LoDep group. Our data thus provide preliminary evidence that deprivation-related symptoms of ADHD and ASD and low IQ have distinct brain signatures compared to non-deprivation-related symptoms, while this hypothesis should be tested further in deprived and non-deprived individuals who meet diagnostic criteria for the respective disorders.
5.4.1 ADHD symptoms

ERA has previously suggested that deprivation-related ADHD symptoms are distinctive in its presentation, persistence and patterns of clinical correlates (Kennedy et al., 2016). The present data suggest that they might also be distinctive neurobiologically. The most striking findings related to cortical gyrification. Most cross-sectional studies of normative clinical samples found no evidence of altered gyrification in patients with ADHD (Forde et al., 2017; Shaw et al., 2012). Consistent with this, in most of the regions that showed a significant interaction between deprivation status and ADHD symptoms on gyrification, there was no significant association in our LoDep group. The one notable exception to this pattern was in the rostral middle frontal cortex, where ADHD symptoms were negatively correlated with gyrification in the LoDep group. Strikingly, of the brain regions identified here, this is the only region that has previously been reported to show reduced gyrification in patients with ADHD (Ambrosino et al., 2017). These findings for the group of adoptees who experienced no or low deprivation are therefore in line with findings from studies examining idiopathic forms of ADHD. In marked contrast, in the Rom>6 group, ADHD symptoms were positively correlated with gyrification in the supramarginal, superior frontal and lingual gyri. Interestingly, these are comparatively late developing cortical regions that show particularly marked increases in cortical folding in the first year of life (Li et al., 2014) – perhaps making them especially vulnerable to adversity in the early postnatal period. Functionally these regions have been associated with a diverse set of processes, from proprioception (supramarginal cortex; Kheradmand, Lasker, & Zee, 2013), over visual processing (lingual cortex; Lee, Hong, Seo, Tae, & Hong, 2000) to attention (superior frontal cortex; Ptak, 2012). It is possible that excessive folding caused by exposure to deprivation represents a neurobiological mediator of the distinctive and
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persistent variant of ADHD seen in the ERA study and similar post-institutionalised samples (Kennedy et al., 2016; Zeanah et al., 2009).

5.4.2 ASD symptoms

Deprivation status also moderated the association between surface area of the left superior parietal cortex and ASD symptoms, with a significant positive association observed in the Rom>6 group only. While activity in this region has been linked to specific ASD symptoms (Travers, Kana, Klinger, Klein, & Klinger, 2015), on a structural level, ASD has been associated with increased overall brain volume in toddlers followed by delayed brain development at later ages (Courchesne, Campbell, & Solso, 2011). No differences in surface area between patients with ASD and healthy controls were found in the mega-analysis by the ENIGMA consortium and another study with a large dataset (Haar, Berman, Behrmann, & Dinstein, 2016; van Rooij et al., 2017). In a longitudinal study, ASD has been associated with an altered developmental trajectories of cortical surface area in multiple brain regions: while it declined with age in adolescent healthy controls, this decline was absent in adolescent patients with ASD (Mensen et al., 2017). However, this effect was not found in the cluster of the left superior parietal cortex reported in this chapter. Our findings are therefore consistent with the idea that deprivation-related ASD symptoms have a distinct brain structural signature. From a neurodevelopmental perspective, the superior parietal cortex is one of the regions that shows the greatest expansion during the first two years of life, with surface area increasing by about 140% in the first two years of life (Lyall et al., 2015). Again, this dramatic increase might make this brain region particularly vulnerable to the deleterious effects of early institutional deprivation. Functionally, this region has been implicated in attentional processing (Ptak, 2012).
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5.4.3 IQ

Previous reports of this study have shown a strong initial negative effect of institutionalisation on IQ, with substantial higher risk of cognitive impairment (IQ < 80) in Rom>6 (Rutter & O’Connor, 2004). However, while other neurodevelopmental problems showed high persistence throughout development, the strength of the effect of institutionalisation on cognitive impairment declined with age. By young adulthood, Rom>6 did not show a significant difference in cognitive impairment rates compared to UK and Rom<6 (Sonuga-Barke et al., 2017), even though overall IQ was still lower on average in Rom>6 (see 4.3.2). This decline in effect suggests environmentally-mediated recovery is possible. This is in contrast to studies investigating the general population, which suggest that IQ is highly heritable and stable from age 7 onwards (Plomin & Deary, 2015; Schneider et al., 2014). Therefore, it was of interest to test whether the neuroanatomical correlates of deprivation-related IQ were the same or different from normative IQ. Here we found that deprivation status significantly moderated the relationship between IQ and brain structure in multiple regions and measures. In Rom>6, IQ was positively related to surface area and volume of the left lingual cortex, while this association was negative and non-significant in LoDep. Moreover, in Rom>6, IQ was negatively related to thickness of the left caudal middle frontal cortex and volume of the right superior parietal cortex, while these relationships were significantly positive in LoDep. The lingual cortex is not commonly associated with intelligence in normative samples but is more commonly found to be related to visual processing (Lee et al., 2000). On the other hand, positive correlations between volumes of the latter two brain regions and IQ have been reported consistently (Basten, Hilger, & Fiebach, 2015) and are in line with the parieto-frontal integration theory of intelligence (Jung & Haier, 2007). The negative relationship between IQ and these two regions in the
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Rom>6 group supports the notion of distinctive structural correlates of IQ in individuals who experienced extended early deprivation compared to those with no or limited deprivation.

5.4.4 Strengths and limitations

This chapter had several strengths. It explored brain structural correlates of deprivation-related problems on a comprehensive whole brain level rather than focusing on a few regions of interest. This allowed regions that have not previously been associated with these neurodevelopmental outcomes to be identified such as the relationship between IQ and the left lingual cortex in Rom>6. At the same time a stringent control for multiple comparisons reduced risk for false positive findings. Moreover, instead of focusing on just one measure such as volume, different measures were investigated. Again, this led to new insights such as the relationship between ADHD symptoms and increased gyrification in multiple brain regions in Rom>6. Only few studies have investigated alterations in local gyrification with regard to early maltreatment or ADHD symptoms and this study suggests a potential link that should be further investigated.

However, this chapter also had limitations. First, this chapter explored neurodevelopmental problems on a symptom rather than on a disorder level. While this continuous approach allows to establish dose-dependent relationships, we are limited in making inferences about individuals who meet diagnostic criteria for disorders. Future studies are needed to compare brain structural correlates in deprived and non-deprived individuals who meet the criteria for a clinical diagnosis of ADHD and ASD, respectively. Relatedly, it should be noted that we had different levels and ranges of symptoms in LoDep and Rom>6, potentially reducing our power to detect interactions between symptoms and brain structure. Particularly,
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there were very low ASD symptom levels in LoDep and therefore results regarding ASD symptoms following no or limited deprivation should be interpreted with caution.

5.4.5 Conclusion

Consistent with the hypothesis that neurodevelopmental disorders emerging following deprivation have a distinctive pathogenesis, we found that deprivation moderated the relationship between neurodevelopmental symptoms and measures of cortical structure in a number of ways. One of the most striking findings was that multiple clusters across the cortex showed a significant interaction between deprivation status and ADHD symptoms on gyrification. In Rom>6, ADHD symptoms were positively associated with gyrification, a relationship that was not seen in LoDep and is also not commonly reported in clinical studies of ADHD. Likewise, in Rom>6 only, ASD symptoms were positively associated with surface area of the left superior parietal gyrus, a region not previously linked to ASD in clinical studies. For IQ, consistent with studies of the general population, there was a positive correlation between IQ and parietal and frontal regions in LoDep but this relationship was reversed in Rom>6. Together, these findings provide preliminary evidence for the hypothesis that deprivation-related forms of ADHD, ASD and cognitive impairment may have distinct neuroanatomical signatures compared to idiopathic variants. Future studies that include deprived and non-deprived participants with clinical diagnoses of these disorders are necessary to further investigate this hypothesis.
CHAPTER 6

STRUCTURAL COVARIANCE OF CORTICAL THICKNESS AND SURFACE AREA IN YOUNG ADULTS WHO EXPERIENCED EARLY DEPRIVATION
ABSTRACT

Brain regions that follow similar developmental trajectories tend to covary in measures such as cortical thickness and surface area. Early environmental adversity may alter such maturational coupling during development. Structural covariance measured in adulthood provides the opportunity to study potential alterations in coordinated brain network development. This study aimed to test whether early childhood institutional deprivation was associated with changes in structural covariance of cortical thickness and surface area in adulthood. We compared 67 Romanian adoptees who had been exposed to either limited or extended institutional deprivation to 21 UK adoptees without deprivation exposure. Early institutional deprivation was associated with long-term alterations in structural covariance, with the most prominent alterations observed following extended deprivation. Most differences in correlation strengths were found between frontal and temporal regions in the right hemisphere, including regions that have previously been shown to be sensitive to institutional deprivation such as the right anterior cingulate cortex and inferior temporal cortex. Cortical thickness and surface area thereby exhibited a distinct pattern of changes, stressing the importance of studying both measures separately. These findings are consistent with the theory that early deprivation permanently alters the coordinated development of brain networks in a pattern that has previously been associated with psychopathology such as ASD and ADHD and which might have been caused by underlying changes in functional connectivity and white matter pathways during development.
6.1 **INTRODUCTION**

Regional characteristics of brain structure are known to covary – with, for instance, cortical thickness and surface area correlated inter-individually across brain regions (Alexander-Bloch, Giedd, & Bullmore, 2013). Structural covariance is thought to result in part from maturational coupling as guided by functional co-activation early in life (Geng et al., 2017). In this way, it represents a structural marker of early functional interactions between regions that are known to develop together (Alexander-Bloch, Raznahan, et al., 2013; Geng et al., 2017; Khundrakpam et al., 2017; Raznahan et al., 2011). Genetic factors are important in regulating processes of covariation within the brain (Schmitt, Giedd, Raznahan, & Neale, 2018; Schmitt et al., 2009). Such effects might explain the recent evidence of altered structural covariance in neurodevelopmental conditions such as autism spectrum disorder (ASD; Balardin et al., 2015; Sharda et al., 2017), attention-deficit/ hyperactivity disorder (ADHD; Bethlehem, Romero-Garcia, Mak, Bullmore, & Baron-Cohen, 2017; Li, Cao, et al., 2015) and conduct disorder (Fairchild et al., 2016). However, longitudinal studies also suggest that patterns of covariation might be influenced by environmental exposures (Karpati, Giacosa, Foster, Penhune, & Hyde, 2018; Romero-Garcia et al., 2018; Schmitt et al., 2018; Schmitt et al., 2009). Given this, studies of structural covariance are potentially a powerful way of identifying the effects of early childhood exposure to environmental adversity on brain development.

Institutional deprivation in early childhood can have long term adverse effects on development and mental health that can be observed even in adulthood (Sonuga-Barke et al., 2017; Zeanah & Sonuga-Barke, 2016). One explanation for the enduring nature of these effects is that such exposures affect brain development during sensitive periods characterised by rapid development and high plasticity.
Impact of early deprivation on adult structural covariance

(Ismail et al., 2017). In support of this view, early maltreatment has been associated with altered structural covariance of cortical thickness in multiple regions (Sun et al., 2018; Teicher et al., 2014). Teicher et al. (2014) reported altered structural covariation across multiple brain regions in adults maltreated in childhood – the anterior cingulate cortex showed reduced connectedness (as measured by centrality), while right precuneus and right anterior insula showed increased connectedness to other brain regions. Sun et al. (2018) found that early maltreatment was related to reduced connectedness in the right temporal pole and increased connectedness in the left supramarginal gyrus. While the former effect was apparent in maltreated youth independent of whether they had developed post-traumatic stress disorder (PTSD), the latter was specific to those without PTSD - highlighting the potential psychopathological specificity of effects (Sun et al., 2018).

While such findings have biological plausibility and potential clinical significance, one has to be cautious about inferring causality - given the design and methodological limitations of the studies on which they are based. Because maltreatment typically occurs within families, the link between exposure to maltreatment and child characteristics (such a brain development) may be confounded by genetic effects on brain development or genetic risk for disorder (Rutter et al., 2012). Moreover, retrospective reports of maltreatment, on which many studies rely, might lead to an oversampling of individuals with psychopathology making it hard to distinguish effects of psychopathology and the effects of deprivation (Hardt & Rutter, 2004).

The English and Romanian Adoptees (ERA) Study is a longitudinal prospective study of the effects of severe maltreatment on development, which overcomes some of these limitations because it capitalises on a natural experiment (Rutter et al., 2012). The study examines individuals who experienced severe deprivation for
up to the first 41 months of their lives in the Romanian institutions of the Ceaușescu regime. Their subsequent adoption into UK families led to a dramatic and precisely timed change from a highly depriving to a mostly loving and nurturing environment. Crucially, the timing of this transition was largely determined by historical and chance events (the fall of the Ceaușescu regime) - so that the extent of deprivation was unlikely to be influenced by genetic factors (Rutter & O’Connor, 2004). As a comparison group, the ERA study also included non-deprived individuals who were adopted within the UK, the vast majority shortly after birth. The latest follow-up of the ERA study also included magnetic resonance imaging (MRI) scanning and therefore allowed us to examine how early institutional deprivation impacts on adult brain structure. In Chapter 3, we have shown that early institutional deprivation is associated with profound loss of total brain volume which is linearly related to the duration of deprivation. Beyond this global effect, there were more subtle differences in brain structure with relatively preserved cortical surface area, thickness and volume in the right inferior temporal cortex following institutionalisation, relatively smaller surface area and volume in the right inferior frontal cortex and a positive association between deprivation duration and surface area and volume in the right medial prefrontal cortex.

Here, we describe the first study of how institutional deprivation in childhood impacts on structural covariance of cortical thickness and surface area in young adulthood. We investigated cortical thickness and surface area separately, as both measures are genetically unrelated (Panizzon, Fennema-Notestine, Eyler, Jernigan, Prom-Wormley, Neale, Jacobson, Lyons, Grant, Franz, et al., 2009), follow distinct developmental trajectories (Wierenga et al., 2014) and their respective structural covariance maps show little overlap in adulthood (Sanabria-Diaz et al., 2010). Investigating cortical volume alone, which is the product of thickness and surface
Impact of early deprivation on adult structural covariance

area, might therefore conflate two measures under distinct genetic and maturational influences (Bethlehem et al., 2017).

Deprivation-related structural covariance alterations would likely be the result of disruptions or adaptations in the coordinated development of brain networks during or following exposure, which cannot be identified by investigating specific structures separately (Bethlehem et al., 2017). Structural covariance analyses provide a tool to investigate network-like properties of the brain rather than isolated regions. Studying changes in structural covariance allows inferences on alterations in maturational coupling, i.e. developmental processes, which can normally not be investigated using cross-sectional data.

We therefore conducted whole brain structural covariance analyses of cortical thickness and cortical surface area. Most studies of structural covariance have focused on cortical thickness only and this is the first study investigating both cortical thickness and surface area in individuals who experienced early maltreatment. In contrast to previous chapters, this analytical approach is limited to categorical group comparisons and does not allow the investigation of continuous factors such as deprivation duration (Alexander-Bloch, Giedd, et al., 2013). To examine the effect of institutionalisation per se as well as deprivation duration, we compared three groups: UK adoptees, who did not experience any institutional deprivation; Romanian adoptees with limited deprivation exposure (< 6 months) and Romanian adoptees with extended deprivation exposure (>6 months). We chose 6 months of deprivation as a cut-off to designate the change from limited to extended deprivation as it has been consistently linked to a significant step-wise increase in the persisting and distinctive pattern of deprivation-related neurodevelopmental impairment (Sonuga-Barke et al., 2017). However, as this analysis was purely based on brain measures and not neurodevelopmental symptoms, we conducted
sensitivity analyses with other potential cut-offs for deprivation duration to confirm the appropriateness of this threshold.

For each group and each measure (cortical thickness and surface area) separately, we used an atlas-based approach to calculate correlation maps across cortical regions. For each group, we calculated (1) the total number of significant correlations between pairs of brain regions across the cortex and (2) the average global correlation strength if considering all correlations, positive correlations only or negative correlations only. We then performed pairwise group comparisons for these measures. Next, in pairwise comparisons, (3) we tested whether groups differed in correlation strength between any specific pair of brain regions or (4) whether there were clusters of brain regions that showed group differences in correlation strength. Finally, we conducted (5) seed-based (rather than atlas-based) analyses to determine whether the clusters previously identified as being sensitive to institutional deprivation in Chapter 3 displayed group differences in correlation strength with other cortical regions.

The total number of significant correlations decreases throughout development (Khundrakpam et al., 2013), indicating differentiation and specialisation of brain regions. Our hypothesis therefore was that early institutional deprivation might be linked to a disruption of this process resulting in a drastic alteration of the total number of significant correlations across the cortex as has been shown in neurodevelopmental disorders such as conduct disorder (Fairchild et al., 2016). An acceleration of brain network specialisation following institutional deprivation would be associated with fewer significant correlations, while a reduced, delayed or halted process of specialisation would be linked to a larger number of significant correlations. For changes in correlation strength between specific brain regions, we predicted that ipsilateral (same hemisphere) areas of the frontal, parietal and
Impact of early deprivation on adult structural covariance
temporal association cortices might show the strongest alterations. These regions
and the white matter pathways that connect them are among the latest to develop,
and structural covariance patterns are determined by both processes (Dubois et al.,
2014; Khundrakpam et al., 2013). Consequently, the inter-correlations between
these regions in cortical thickness and surface area might be particularly vulnerable
to the effects of early maltreatment.
6.2 METHODS

6.2.1 Participants

Participants were recruited as described in 2.3 and 2.5.1. The same participants that were included in the previous chapters were included in this analysis. Hence, the final sample comprised 67 Romanian adoptees (Rom; 40.6% of the original sample, 50.7% female, mean age=25.3 years, SD age=1.1 years) and 21 UK adoptees (UK, 40% of the original sample, 38.1% female, mean age=24.4 years, SD age=1.0 years). 21 Romanian adoptees had been institutionalised for less than 6 months (Rom<6, 42.9% female, mean age=24.5 years, SD age=0.9 years) and 46 adoptees had been exposed to more than 6 months of institutional deprivation (Rom>6, 54.3% female, mean age=25.7 years, SD age=0.9 years).

6.2.2 Procedure, MRI data acquisition and processing

The procedure and details of MRI acquisition and processing have been described in detail in 2.5 and 2.6. The same T1 structural MR scans (3.0 Tesla) as utilised in the previous chapter were used here. Cortical thickness (CT) and cortical surface area (CSA) were quantified using FreeSurfer 6.0.0.

6.2.3 Structural covariance: atlas-based analysis

6.2.3.1 Network construction

Data were analysed in Matlab 2017a (MathWorks, 1996) and R 3.5.0 (R Core Team, 2018). For the atlas-based analysis, we extracted CSA and average CT values for each of the 68 brain regions defined in the Desikan-Killiany atlas (Figure 6.1; Desikan et al., 2006). We then regressed out sex (mean-centred) using a general linear model to control for sex differences in each of the measures (Ruigrok
et al., 2014). We also controlled for total brain volume (mean-centred) in CSA but not CT, as total brain volume does not scale with CT (Barnes et al., 2010). To create CT and CSA covariance matrices, CT and CSA residuals were correlated for each group separately using Pearson’s product-moment correlation resulting in 2278 correlation ($r$) values for each measure.

Figure 6.1: Cortical regions of the Desikan-Killiany atlas. Each region is displayed in a different colour on an inflated surface. 34 brain regions of the left hemisphere are displayed in lateral (left) and medial view (right).

6.2.3.2  Total number of significant correlations and average correlation strength

The total number of significant correlations per group was established after applying a correction for multiple comparisons using the false discovery rate (FDR) procedure ($q=0.05$, Benjamini & Hochberg, 1995). Average correlation strength was computed for each group by taking all correlations into account as well as positive and negative correlations separately.

We used permutation testing to determine if there were significant group differences in the above measures. 100,000 permutations were performed. For each permutation, participants were randomly assigned to either group while keeping group sample sizes constant. For each permutation, correlation values were calculated and the total number of significant correlations as well as average correlation strengths were computed for each group as described above. A $p$-value
for each measure was calculated by determining its position in the permuted distribution (using absolute values as this was a two-sided test).

6.2.3.3 Differences in correlation strength between pairs of brain regions

To investigate whether there were significant group differences in correlation strength between brain regions, we first Fisher z-transformed all r-values to maximise normality. We then calculated the group difference for each correlation. We used permutation testing as described above to calculate non-parametric probability values for every group difference. One million permutations were performed to allow probability distributions with high enough resolution to allow for multiple comparisons correction. For each permutation, correlation values were z-transformed and their differences were calculated. This resulted in a probability distribution for each difference value against which the true correlation difference could be tested. Differences that were more extreme than 95% of absolute distribution values were deemed statistically significant (two-sided test).

6.2.3.4 Multiple comparisons correction

To control for multiple comparisons, we used two different approaches. In the first approach, FDR correction was applied to all 2278 resulting probability values of the difference matrix. One of the benefits of FDR correction is that it allows one to identify specific pairs of brain regions that show a strong group difference in correlation strength. On the other hand, this difference has to be extreme to survive such stringent correction and potential dependencies between regions in correlation strength are not being considered.

For the second approach, we used the network-based statistic (NBS), which has been described as cluster-wise correction (Zalesky, Fornito, & Bullmore, 2010) and has previously been implemented in studies of structural covariance (Reess et al.,
The advantage of NBS lies in its increased power in situations in which there is a set of connected brain regions (which can be compared to a cluster of connected voxels in fMRI analysis and a component of connected nodes in graph theory) that show group differences in their correlation strength. If this group is larger than expected by chance, it will be statistically significant, even if single connections within it might not pass the stringent threshold of FDR correction. The drawbacks of NBS are that it is only possible to determine the statistical significance for the cluster, rather than each pair of regions within the cluster, and that it relies on an arbitrary definition of a primary threshold (Zalesky et al., 2010). For this analysis, we first used Fisher’s Z Test to compute p values for the group differences between the z-transformed correlation matrixes. We then applied a primary threshold of $p<0.005$ (two-sided) to define a network of brain regions that show potential group differences. Graph theory was then used to define the maximum component size within this network, i.e. the number of connected regions that showed correlations more extreme than the primary threshold. These steps were also added during permutation testing: For each permutation, we calculated the maximum component size for the permuted difference matrix after applying the same primary threshold. We then determined where the true component size lies within the distribution of maximum component sizes. If it was greater than 95% of the distribution, it was deemed statistically significant (one-sided test).

6.2.3.5 Group comparisons

As described in the introduction, the structural covariance analyses described here were limited to group comparisons and did not allow linear regressions with continuous factors such as deprivation duration to be performed. To investigate the effect of institutionalisation per se and deprivation duration, pairwise comparisons were performed for UK, Rom<6 and Rom>6 testing whether limited and extended
deprivation were associated with changes in structural covariance compared to no deprivation and each other.

6.2.4 Structural covariance: seed-based analysis

We used the Mapping Anatomical Correlations Across Cerebral Cortex (MACACC) approach to test whether CT and CSA in our predefined seeds showed different correlations with the rest of the cortex depending on group membership. The MACACC approach was first introduced by Lerch et al. (2006) and has since been used as a seed-based structural covariance approach in many studies (for example: (Karpati et al., 2018; Khundrakpam et al., 2017; Sharda et al., 2017; Sharda, Khundrakpam, Evans, & Singh, 2016). The benefit of MACACC is that it is a whole-brain vertex-wise approach and therefore sensitive to clusters that might not align neatly with anatomical atlas regions (i.e., those derived from the Desikan-Killiany atlas). First, a 10 mm kernel at full-width/ half-max (FWHM) was applied for smoothing CT and CSA values across the cortex. Our CT seed was a cluster in the inferior temporal cortex, which was previously found to show greater CT values following institutionalisation in this cohort. Our SCA seeds were clusters in the (1) inferior temporal cortex, which was previously found to show greater CSA following institutionalisation; (2) inferior frontal cortex, which was previously found to show lower CSA following institutionalisation, and (3) medial prefrontal cortex, in which duration of deprivation was previously shown to positively correlate with CSA (see 3.3.4). We extracted the mean CT or CSA values of these seed regions. We then tested whether there was an interaction between group and seed CT/CSA values on any clusters across the cortex in separate general linear models. A mask was applied that excluded the respective seed region itself from the analysis. As in the atlas-based analysis, we accounted for potential sex differences by entering sex as
a covariate as well as total brain volume for SCA analyses only. Results were corrected for multiple comparisons using a Monte Carlo simulation (vertexwise threshold $p<0.05$, clusterwise threshold $p<0.05$). For significant clusters, we extracted the average vertex-wise CT/CSA value of each participant and correlated these with individual seed CT/CSA values within each group after partialling out sex for CT and CSA and total brain volume for CSA only (Pearson’s product-moment correlation).

6.2.4.1 Group comparisons

Seed 1 (inferior temporal CT and CSA) and seed 2 (inferior frontal CSA) were previously found to show a difference between UK and Rom. To further test if institutionalisation and deprivation status were associated with changes in correlation strength in these two seeds, we compared Rom<6 and Rom>6 to UK in separate analyses.

Seed 3 (medial prefrontal CSA) showed a linear relationship with deprivation duration within Rom. We therefore compared Rom<6 to Rom>6 to examine if those with extended deprivation show differential structural covariance of this seed compared to those with limited deprivation.

6.2.5 Sensitivity analysis

A limitation of the structural covariance approaches described here is that they rely on categorical group comparisons rather than allowing one to examine the influence of continuous variables such as deprivation duration (Alexander-Bloch, Giedd, et al., 2013). Even though we have strong evidence for a step-wise change in neurodevelopmental symptoms at 6 months deprivation-duration, it was unclear whether this cut-off constitutes a neurobiologically meaningful threshold in terms of differences in structural covariance. We therefore repeated the analyses above
using different thresholds to test whether they would produce similar results. To ensure that sample sizes per group were at least 20, which is important given statistical power considerations, we were only able to move the threshold higher than 6 months rather than lower. Consequently, moving in 2 months steps, the limited/extended deprivation groups were defined as having been institutionalised for less/more than 8, 10 or 12 months, respectively (limited deprivation: Rom<8, Rom<10, Rom<12 (range n: 23 – 29); extended deprivation: Rom>8, Rom>10, Rom>12 (range n: 38 – 44)).

6.2.5.1 Atlas-based analyses

In pairwise comparisons, we tested for differences between the UK group, the limited deprivation group and the extended deprivation group (UK vs Rom<8 vs Rom>8; UK vs Rom<10 vs Rom>10; UK vs Rom<12 vs Rom>12).

6.2.5.2 Seed-based analyses

For seed 1 (inferior temporal CT and CSA) and seed 2 (inferior frontal CSA) we compared the UK group to the limited deprivation group and the extended deprivation group, respectively (UK vs Rom<8; UK vs Rom>8; UK vs Rom<10; UK vs Rom>10; UK vs Rom<12; UK vs Rom>12).

For seed 3 (medial prefrontal SCA) we tested for group differences between the limited and extended deprivation group (Rom<8 vs Rom>8; Rom<10 vs Rom>10; Rom<12; Rom>12).
6.3 RESULTS

6.3.1 Cortical thickness

6.3.1.1 Atlas-based analysis

General pattern, average correlation strength and total number of significant correlations

The total number of significant correlations and average correlation strengths per group are displayed in Figure 6.2. The upper panel of Figure 6.2 (a, b, c) shows that in all groups, CT values across regions were mostly positively correlated. In fact, only one negative correlation was significant after FDR correction: the right rostral anterior cingulate cortex and right inferior temporal cortex were negatively correlated and this was only the case in the UK group ($r=-0.71$, $p_{FDR}<0.05$). Descriptively, cingulate and temporal regions were generally negatively correlated within UK in both ipsilateral and contralateral (opposite hemisphere) CT with stronger correlations in the right hemisphere (Figure 6.2a). In contrast, there was a mix of positive and negative correlations in the Rom<6 group (Figure 6.2b), and mostly positive correlations observed in the Rom>6 group (Figure 6.2c). A similar pattern was found for correlation strength between ipsi- and contralateral cingulate and occipital regions.

Other lobes showed more similar patterns across groups: There were strongly positive ipsi- and contralateral correlations between fronto-parietal and parieto-occipital lobes. The strongest positive correlations in all groups were between homotopic areas (the same regions in contralateral hemispheres, indicated by strongly positively correlated diagonal lines in Figure 6.2 a, b, c) and ipsi- and contralateral correlations within parietal lobes.
Descriptively, the negative correlations in Rom>6 were less strong. However, there were no significant differences between any of the groups in the average strength for all correlations (all $p>0.69$), for positive correlations only (all $p>0.21$) or negative correlations only (all $p>0.20$). Likewise, even though Rom>6 showed a higher total number of significant correlations than the other two groups, these differences were not significant (all $p>0.54$). This suggests that more correlations survive FDR correction in the Rom>6 group because of a larger sample size in this group and associated lower $p$-values.

**Differences in correlation strength between pairs of brain regions**

In CT structural covariance, differences between UK and Rom>6 were seen in correlation strength between two pairs of brain regions. First, the correlation strength between the right rostral anterior cingulate cortex and right inferior temporal cortex differed between groups ($p_{FDR}<0.05$) with a strong negative correlation in UK ($r=-0.71$, $p_{FDR}<0.05$) and a positive correlation in Rom>6 ($r=0.46$, $p_{FDR}<0.01$, Table 6.1, Figure 6.2, Figure 6.3a). Second, the correlation between the right frontal pole and right transverse temporal cortex was significantly stronger in UK ($r=0.77$, $p_{FDR}<0.01$) than Rom>6 ($r=-0.08$, $p_{FDR}=0.69$; group difference: $p_{FDR}<0.05$, Table 6.1, Figure 6.2, Figure 6.3b). There were no significant differences in correlation strength between UK and Rom<6 and no differences between Rom<6 and Rom>6. When using NBS instead of FDR correction to identify clusters of connected sets of regions that showed alterations in correlation strength, maximum component size did not reach statistical significance for any group comparison (all $p>0.41$).
Table 6.1: Cortical thickness atlas-based covariance results. Regions showing significant group differences in their correlation strengths. The difference was calculated after z-transforming the correlation coefficients.

<table>
<thead>
<tr>
<th>region A</th>
<th>region B</th>
<th>$r$</th>
<th>$p_{FDR}$</th>
<th>$r$</th>
<th>$p_{FDR}$</th>
<th>difference (z-transformed)</th>
<th>$p_{FDR}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>right frontal pole</td>
<td>right transverse temporal</td>
<td>0.77</td>
<td>0.003</td>
<td>-0.08</td>
<td>0.689</td>
<td>-1.11</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>right rostral anterior cingulate</td>
<td>-0.71</td>
<td>0.010</td>
<td>0.46</td>
<td>0.009</td>
<td>1.39</td>
<td>0.027</td>
</tr>
</tbody>
</table>

$r$ – Pearson’s correlation coefficient, UK – UK adoptee group, Rom>6 – Romanian adoptees with more than 6 months of deprivation duration
Impact of early deprivation on adult structural covariance

Figure 6.2: Cortical thickness covariance matrices. Top row: Strength maps (blue: negative, red: positive) for all correlations between brain regions (bottom half of each matrix) or only significant cross-cortical correlations (upper half of each matrix) in UK, Rom<6 and Rom>6 (left to right). The header of this row displays the total number of significant correlations for each group as well as average correlation strength for all correlations, positive only correlations and negative only correlations (irrespective of significance level). Bottom row: maps showing differences in correlation strength between UK vs Rom<6 (left), UK vs Rom>6 (middle) and Rom<6 vs Rom>6 groups (right; after z-transformation, blue: more negative correlations in first named group, red: more positive correlations in first named group). The lower half of each matrix shows all differences (including the direction of the effects), while the upper half only shows significant ones.

6.3.1.2 Seed-based analysis

Results of the seed-based analysis partly confirmed those of the atlas-based analysis. UK and Rom>6 showed significantly different correlation strength between CT in the inferior temporal seed region and CT in a cluster including the right rostral
and caudal anterior cingulate cortex ($p_{\text{cluster}}<0.05$, Table 6.2, Figure 6.3c). In UK, this correlation was strongly negative, while it was strongly positive in Rom>6.

**Table 6.2: Cortical thickness seed-based covariance results.** Cluster that showed a significant group difference in correlation strength with the seed region (located in the right inferior temporal cortex) in terms of CT (vertexwise threshold $p<0.05$, clusterwise threshold $p<0.05$).

<table>
<thead>
<tr>
<th>group 1</th>
<th>group 2</th>
<th>region</th>
<th>H</th>
<th>Cluster size [mm$^2$]</th>
<th>Peak MNI coordinates [mm]</th>
<th>Cluster-wise</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>Rom&gt;6</td>
<td>rostral anterior cingulate</td>
<td>L</td>
<td>916</td>
<td>13 37 13</td>
<td>0.01</td>
<td>UK: $r=-0.56^{<strong>}$ Rom&gt;6: $r=0.50^{</strong>*}$</td>
</tr>
</tbody>
</table>

H – hemisphere, L – left, R – right, r – partial Pearson’s correlation coefficient, UK – UK adoptee group, Rom>6 – Romanian adoptees with more than 6 months of deprivation duration
6.3.2 Cortical surface area

6.3.2.1 Atlas-based analysis

General pattern, average correlation strength and total number of significant correlations

The total number of significant correlations and average correlation strength per group are displayed in Figure 6.4. Again, even though descriptively Rom>6 showed the highest total number of significant correlations after FDR-correction, the group differences did not reach statistical significance (all $p>0.54$), possibly indicating that the higher total number in Rom>6 was driven by differences in sample size.
There were also no significant group differences in the average strength across all correlations (all $p>0.69$), positive-only correlations (all $p>0.21$) or negative-only correlations (all $p>0.20$). Compared to the CT correlation matrices, CSA maps were more diffuse with many positive and negative correlations in all groups and no clear correlation patterns across ipsi- and contralateral lobes or homotopic areas. This is corroborated by low average strength across all correlations. Still, most of the correlations that survived FDR correction were positive, with only one significant negative correlation between the left medial orbitofrontal cortex and left insula in UK ($r=-0.72$, $p_{FDR}<0.05$).

*Differences in correlation strength between pairs of brain regions*

There were no significant group differences in correlation strength between any two brain regions in CSA with either FDR correction (all $p_{FDR}>0.29$, Figure 6.4, bottom panel) or using the NBS approach (all $p>0.71$).
Figure 6.4: Cortical surface area covariance matrices. Top row: Strength maps (blue: negative, red: positive) for all correlations between brain regions (bottom half of each matrix) or only significant correlations (upper half of each matrix) in UK, Rom<6 and Rom>6 (left to right). Bottom row: difference maps between UK vs Rom<6 (left), UK vs Rom>6 (middle) and Rom<6 vs Rom>6 (right; after z-transformation, blue: more negative correlations in first named group, red: more positive correlations in first named group). The lower half of each matrix shows all differences, regardless of direction, while the upper half only shows significant inter-regional correlations.

### 6.3.2.2 Seed-based analysis

Results of the seed-based CSA covariance analysis are summarized in Table 6.3 and Figure 6.5.

**Seed 1: inferior temporal**

The correlation between inferior temporal seed CSA and a cluster in the right superior frontal cortex differed significantly between UK and Rom>6 groups.
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\(p_{\text{cluster}}<0.05\), with a negative correlation in UK but a positive correlation in Rom>6. The same seed region also showed a significant group difference in correlation strength with a cluster in the temporo-parietal junction between UK and Rom<6 \(p_{\text{cluster}}<0.05\). This correlation was negative in UK and positive in Rom>6 (but non-significant when groups were considered separately).

**Seed 2: inferior frontal**

There was a significant difference between UK and Rom>6 in CSA correlation strength between the inferior frontal seed region and a cluster in the left inferior temporal cortex \(p_{\text{cluster}}<0.05\). In UK, this correlation was positive, while it was and negative (albeit non-significant) in Rom>6.

**Seed 3: medial prefrontal**

There were two clusters that differed between Rom<6 and Rom>6 in correlation strength to the medial prefrontal seed (both \(p_{\text{cluster}}<0.01\)). The right fusiform cortex was positively correlated with this seed in Rom>6, but negatively correlated in Rom<6. On the other hand, the right caudal middle frontal cortex was positively correlated with the medial prefrontal cortex seed in Rom<6, but there was no significant correlation in Rom>6.
## Table 6.3: Cortical surface area seed-based covariance results

Clusters that showed a significant group difference in correlation strength with the seed regions in terms of CSA (vertexwise threshold $p<0.05$, clusterwise threshold $p<0.05$).

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Region</th>
<th>Hemisphere</th>
<th>Cluster size [mm$^2$]</th>
<th>Peak MNI coordinates [mm]</th>
<th>Clusterwise Effect Sizes</th>
<th>Partial Pearson's correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>seed 1: inferior temporal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Rom&gt;6</td>
<td>superior frontal</td>
<td>R</td>
<td>2562</td>
<td>20 39 34</td>
<td>0.0002</td>
<td>UK: $r=-0.64^{**}$ Rom&gt;6: $r=0.31^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>temporo-parietal</td>
<td>R</td>
<td>1447</td>
<td>63 -36 10</td>
<td>0.0406</td>
<td>UK: $r=-0.38$ Rom&lt;6: $r=0.34$</td>
</tr>
<tr>
<td><strong>seed 2: inferior frontal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Rom&gt;6</td>
<td>inferior temporal</td>
<td>L</td>
<td>1593</td>
<td>-54 -23 -32</td>
<td>0.0219</td>
<td>UK: $r=0.57^{**}$ Rom&gt;6: $r=-0.26$</td>
</tr>
<tr>
<td><strong>seed 3: medial prefrontal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rom&lt;6</td>
<td>Rom&gt;6</td>
<td>fusiform</td>
<td>R</td>
<td>1829</td>
<td>34 -68 -14</td>
<td>0.0080</td>
<td>Rom&lt;6: $r=-0.47^{<strong>}$ Rom&gt;6: $r=0.40^{</strong>}$</td>
</tr>
<tr>
<td>Rom&lt;6</td>
<td>Rom&gt;6</td>
<td>caudal middle frontal</td>
<td>R</td>
<td>1861</td>
<td>36 12 54</td>
<td>0.0068</td>
<td>Rom&lt;6: $r=0.60^{**}$ Rom&gt;6: $r=0.05$</td>
</tr>
</tbody>
</table>

$H$ – hemisphere, $L$ – left, $R$ – right, $r$ – partial Pearson’s correlation coefficient, UK – UK adoptee group, Rom<6 – Romanian adoptees with less than 6 months of deprivation duration, Rom>6 – Romanian adoptees with more than 6 months of deprivation duration
Figure 6.5: Summary of cortical surface area (CSA) seed-based covariance findings. a) Significant difference between UK (red regression line) and Rom>6 (orange regression line) in the correlation between seed 1 (right inferior temporal, blue region) and right superior frontal CSA (yellow region). Significant difference between UK and Rom<6 (purple regression line) in correlation between seed 1 and right temporo-parietal junction (red region). b) Significant difference between UK and Rom>6 in the correlation between seed 2 (inferior frontal, blue region) and left inferior temporal CSA (yellow region). c) Significant difference between Rom<6 and Rom>6 in correlation between seed 3 (medial prefrontal, blue region) and right fusiform (orange region, left) and right caudal middle frontal (orange region, right) cortices. For each graph, shaded areas depict 95% confidence intervals around the regression line and dots represent individual data points after regressing out the effects of sex and total brain volume.

6.3.3 Sensitivity analysis

To investigate whether a cut-off at 6 months deprivation duration was appropriate for the limited and extended deprivation distinction in Rom, we performed sensitivity analyses. These were replications of the above analyses, but with slightly altered deprivation duration thresholds for both Rom groups.

In the atlas-based analyses, the differences between the UK and extended deprivation groups remained significant remained significant at higher thresholds of
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8 months and 10 months but not at 12 months (most likely due to reduced power, Table 6.4). This indicates that a 6 months threshold was equally sensitive to detect alterations associated with extended deprivation as higher thresholds of 8 or 10 months.

Table 6.4: Sensitivity analyses for CT atlas-based covariance results. Pairs of regions showing significant group differences in their correlation strength. The difference values were calculated after z-transforming the correlation coefficients.

<table>
<thead>
<tr>
<th>region A</th>
<th>region B</th>
<th>( r )</th>
<th>( p_{FDR} )</th>
<th>( r )</th>
<th>( p_{FDR} )</th>
<th>Difference</th>
<th>( p_{FDR} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>Rom &lt; 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no significant differences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Rom &lt; 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no significant differences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>Rom &lt; 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right inferior temporal</td>
<td>right rostral anterior cingulate</td>
<td>-0.73</td>
<td>0.009</td>
<td>0.64</td>
<td>0.007</td>
<td>-1.69</td>
<td>0.046</td>
</tr>
<tr>
<td>UK</td>
<td>Rom &gt; 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right frontal pole</td>
<td>right transverse temporal</td>
<td>0.77</td>
<td>0.003</td>
<td>-0.09</td>
<td>0.667</td>
<td>1.12</td>
<td>0.026</td>
</tr>
<tr>
<td>right inferior temporal</td>
<td>right rostral anterior cingulate</td>
<td>-0.71</td>
<td>0.010</td>
<td>0.47</td>
<td>0.009</td>
<td>-1.40</td>
<td>0.025</td>
</tr>
<tr>
<td>UK</td>
<td>Rom &gt; 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right frontal pole</td>
<td>right transverse temporal</td>
<td>0.77</td>
<td>0.003</td>
<td>-0.10</td>
<td>0.610</td>
<td>-1.11</td>
<td>1.131</td>
</tr>
<tr>
<td>right inferior temporal</td>
<td>right rostral anterior cingulate</td>
<td>-0.70</td>
<td>0.011</td>
<td>0.49</td>
<td>0.009</td>
<td>1.39</td>
<td>1.400</td>
</tr>
<tr>
<td>UK</td>
<td>Rom &gt; 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no significant differences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rom &lt; 8</td>
<td>Rom &gt; 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no significant differences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rom &lt; 10</td>
<td>Rom &gt; 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no significant differences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rom &lt; 12</td>
<td>Rom &gt; 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no significant differences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

H – hemisphere, L – left, R – right, \( r \) – partial Pearson’s correlation coefficient

For the seed-based analyses, there was evidence that the effects observed in the limited deprivation group were sensitive to deprivation duration threshold alterations (Table 6.5). Effects seen in this group did not remain significant if the threshold for deprivation duration was set higher. This suggests that a cut-off at 6 months
deprivation duration is justified to identify effects that are specific to limited deprivation. On the other hand, effects observed in the extended deprivation group were largely robust to changes in the deprivation duration threshold and most remained significant if this threshold was set higher. Importantly, a cut-off at 6 months seemed as sensitive as higher thresholds as the new analyses identified clusters in similar locations, and across all analyses only two new clusters were found (when assuming a 12-month threshold, marked in grey in Table 6.5).

Together, these data suggest that a cut-off at 6 months deprivation duration might be the most sensitive to detect the effects of limited deprivation and is as sensitive as other higher thresholds in terms of detecting the effects of extended deprivation.
### Table 6.5: Sensitivity analyses for seed-based covariance results

Clusters that showed a significant group difference in correlation strength with the seed regions in terms of CT/CSA (vertexwise threshold $p<0.05$, clusterwise threshold $p<0.05$). Grey areas highlight novel findings that were not observed when using a 6 months threshold to designate extended deprivation.

<table>
<thead>
<tr>
<th>group 1</th>
<th>group 2</th>
<th>region</th>
<th>H</th>
<th>clust size [mm$^2$]</th>
<th>Peak MNI coordinates [mm]</th>
<th>clusterwise $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$x$ $y$ $z$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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#### Seed 1: Inferior Temporal

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Impact of early deprivation on adult structural covariance
6.4 DISCUSSION

Here we have shown that early childhood institutional deprivation is associated with adult alterations in structural covariance between cortical areas as measured by inter-regional correlations of cortical thickness and surface area. These findings are consistent with the notion that regional effects in these measures as identified in Chapter 3 do not represent isolated morphometric alterations but are in fact associated with changes in structural covariance to other brain regions. We interpret this as evidence for institutional deprivation disrupting the maturational coupling of brain networks during development (Alexander-Bloch, Raznahan, et al., 2013). Changes in structural covariance thereby followed a particular pattern.

First, nearly all group differences in correlation strength were found between frontal and temporal regions (if the anterior cingulate cortex is considered frontal). The notable exception was altered fronto-frontal correlation strength between the right medial prefrontal cortex and right rostral middle frontal cortex when comparing Rom<6 and Rom>6. This partly confirms our hypothesis that the strongest alterations in structural covariance measures would be found in frontal, parietal and temporal association cortices.

Second, most differences in correlation strength were limited to right ipsilateral regions, with the only exception seen in altered covariation in surface area between the right inferior frontal cortex and left inferior temporal cortex when comparing UK and Rom>6. This pattern did not only emerge in the seed-based analyses but was also evident in the atlas-based approach for cortical thickness. This suggests that the coupling between regions in the right hemisphere might be more malleable to restructuring following early institutional deprivation, while the left hemisphere might be more resilient.
Third, there were group differences in correlation strength (particularly for UK vs. Rom>6) for both cortical thickness and surface area, but the regions implicated in each analysis were different. Only one difference was found in adjacent areas for cortical thickness and surface area: Right inferior temporal cortex and anterior cingulate cortex showed a more positive correlation in Rom>6 compared to UK in cortical thickness. The same pattern was found for right inferior temporal cortex and right superior frontal cortex in surface area. This suggests that cortical thickness and surface area structural covariance is differentially affected by institutional deprivation, most likely due to the fact that these measures display different developmental trajectories (Wierenga et al., 2014) and resulting differences in time windows of heightened plasticity and vulnerability. These findings stress the importance of considering both measures separately.

Fourth, contrary to our hypothesis, we found no significant group differences in the total number of significant correlations, indicating that the alterations in structural covariance were limited to the specific regions identified here rather than representing a disruption of structural covariance on a global brain level. Moreover, most differences were found when comparing UK and Rom>6, suggesting that individuals with extended deprivation show greater differences in structural covariance than those with limited deprivation. An illustration of the main differences in structural covariance can be found in Figure 6.6.
Figure 6.6: Main structural covariance findings. Highlighting changes in structural covariance in Rom>6 compared to either UK or Rom<6. Group differences in structural covariance of cortical thickness (a, b) and surface area (c, d) in axial (a, c) and sagittal (b, d) view. Red lines represent increases in correlation strength in Rom>6, while blue lines represent decreases. Please note that lines represent changes in correlation strength and do not represent physical connections (such as white matter) between regions. For atlas-based analyses, the centre of a brain region according to the atlas is displayed (black dot). For seed-based analyses, peak coordinates of significant clusters were chosen.

6.4.1 Altered fronto-temporal structural covariance following institutional deprivation

In Rom>6, there were stronger and more positive correlations between right inferior temporal cortex and anterior cingulate cortex (cortical thickness, compared to UK), right inferior temporal cortex and right superior frontal cortex (cortical surface area, compared to UK) and right fusiform cortex and anterior cingulate cortex (cortical surface area, compared to Rom<6). In contrast, correlation strength of cortical
thickness between right frontal pole and right transverse temporal cortex was much lower in Rom>6 compared to the positive correlation observed in the UK group. Descriptively, these findings mirrored a more general pattern in CT covariance maps of increased correlation between right cingulo-temporal regions and reduced correlation between right fronto-temporal regions following extended deprivation, while the pattern for cortical surface area was more heterogeneous. Inferior temporal cortex, fusiform cortex, superior frontal cortex and anterior cingulate cortex have been implicated as sensitive to institutional deprivation in Chapter 3 and it is striking that the correlation strength between inferior temporal cortex and anterior cingulate cortex showed the strongest difference in the whole brain atlas-based approach for cortical thickness. These brain regions have also been shown to be related to neurodevelopmental and neuropsychological outcomes in Chapter 4. We hypothesise that changes in brain-behaviour-correlates following extended deprivation might partly be mediated by changes in structural covariance of these regions.

6.4.2 Structural covariance in previous studies of early maltreatment, early adverse environment and psychopathology

A similar pattern to our findings was reported in a previous study investigating the impact of early adversity: individuals who were born very pre-term also show increased structural covariance between anterior cingulate cortex and inferior temporal cortex (among other regions; Nosarti et al., 2011). Teicher et al. (2014) also found anterior cingulate cortex and inferior temporal cortex to be sensitive to early maltreatment. However in this study these regions showed less centrality, a measure of the degree of connectedness of a brain region, in maltreated individuals (Teicher et al., 2014). We did not measure centrality directly, however, our results
show that the anterior cingulate cortex showed stronger positive deprivation-related correlations with temporal brain regions. Potential reasons why studies have discrepant findings might lie in the different methodological approaches and limitations of previous observational study designs as discussed in the introduction. It will be interesting to apply graph theory measures to this structural covariance analysis to compare findings the study by Teicher et al. (2014) more directly.

With regard to psychopathology, alterations in fronto-parietal-temporal structural covariance have also been reported in children and adults with autism spectrum disorder (Ecker et al., 2013; Sharda et al., 2017). However, ASD was associated with reduced fronto-temporal structural covariance (Sharda et al., 2017) and less connectedness in fronto-parietal regions (Bethlehem et al., 2017). Structural covariance studies in individuals with ADHD are very scarce, but there is evidence for lower connection distance in patients with ADHD (Bethlehem et al., 2017). Whether individuals with a diagnosis of ADHD or ASD differ in structural covariance depending on whether they experienced early maltreatment or not has not been investigated yet.

Previous structural covariance studies find stronger fronto-temporal correlations including the anterior cingulate cortex in patients with psychosis and this pattern has been associated with auditory hallucinations (Alexander-Bloch, Giedd, et al., 2013; Collin et al., 2013; Mitelman, Buchsbaum, Brickman, & Shihabuddin, 2005; Modinos et al., 2009; Sandini et al., 2018; Wible et al., 2001). There is a link between early maltreatment and psychosis (Croft et al., 2018), however, ERA has not found strong evidence for increased rates of psychosis following deprivation (Sonuga-Barke et al., 2017). Future studies investigating structural covariance in patients with psychosis should consider a potential link to early maltreatment experiences. It might be that structural covariance differences attributed to psychopathology are
driven by differences in maltreatment exposure. For example, Kumari et al. (2013) found that anterior cingulate cortex volume differences between patients with schizophrenia and healthy controls could be explained by psychosocial deprivation.

Taken together, there is evidence that abnormalities in fronto-temporal structural covariance are associated with psychopathology, with some evidence in studies investigating ASD and ADHD, and stronger evidence in studies investigating psychosis (which is also the most extensively studied condition in terms of structural covariance; Alexander-Bloch, Giedd, et al., 2013). More research is needed to disentangle the effects of maltreatment and subsequent psychopathology on structural covariance. For example, it would be interesting to compare Romanian adoptees with deprivation-related neurodevelopmental or psychiatric problems to those who are problem-free (i.e. do not show elevated symptoms) to test whether deprivation-related problems are associated with distinct changes in structural covariance.

6.4.3 Potential underlying changes in functional connectivity and white matter tracts

Structural covariance is thought to result in part from maturational coupling – brain regions that develop at the same rate, correlate in anatomical measures such as cortical thickness (Alexander-Bloch, Raznahan, et al., 2013). However, it remains unclear how this link between maturational coupling and structural covariance relates to other forms of brain connectivity, namely white matter connectivity as measured with diffusion tensor imaging (DTI) and functional connectivity, the temporal correlation of brain activity. There is some indication that structural covariance might be guided by functional connectivity in infancy (Geng et al., 2017) leading to a partial overlap between functional connectivity and structural
Impact of early deprivation on adult structural covariance

covariance in adulthood (Segall et al., 2012). This hypothesis also indicates that vulnerability of structural covariance to early adverse experiences might be the result of experience-dependent changes in functional connectivity. Altered functional connectivity following early maltreatment has mostly been reported in fronto-limbic neural circuits associated with emotion regulation (McCrory et al., 2017). These circuits included functional connectivity changes in regions implicated in our study, such as the medial prefrontal cortex and anterior cingulate cortex, even though their covariance to subcortical structures could not be tested using the present approach which focuses on the cortex. Reduced functional connectivity was also found between dorsolateral prefrontal cortex and anterior cingulate cortex in children with early separation experiences during a peer rejection paradigm (Puetz et al., 2014), which is in line with our finding of reduced structural covariance between right middle frontal cortex and anterior cingulate cortex when comparing adoptees with extended versus limited deprivation.

Structural covariance networks also show partial convergence with white matter axonal connections as measured by DTI (Gong et al., 2012) and changes in structural covariance following institutional deprivation might be mirrored by alterations in underlying white matter tracts. The cingulum bundle is a prominent white matter tract that connects anterior cingulate cortex and inferior temporal cortex among other regions (Bubb, Metzler-Baddeley, & Aggleton, 2018). This fibre bundle has been shown to be altered in individuals who were exposed to different forms of early childhood maltreatment including neglect and institutionalisation (Choi, Jeong, Polcari, Rohan, & Teicher, 2012; Hanson et al., 2013; Huang et al., 2012; Kumar et al., 2014; Ugwu, Amico, Carballedo, Fagan, & Frodl, 2015). Moreover, the cingulum bundle was shown to be altered in adoptees exposed to institutional deprivation (Bick et al., 2015). Additional white matter tracts have been
identified by these studies. The arcuate fasciculus, for example, connects the caudal temporal lobe with the dorsolateral prefrontal cortex (Kamali, Flanders, Brody, Hunter, & Hasan, 2014) and has been shown to be altered in individuals exposed to parental verbal abuse or early deprivation (Choi, Jeong, Rohan, Polcari, & Teicher, 2009; Govindan et al., 2010).

In conclusion, changes in structural covariance following early institutional deprivation might partly represent alterations in functional coupling of brain regions. Altered covariance between fronto-frontal surface area following extended deprivation might be related to maltreatment-related alterations in emotion regulation. White matter tracts, such as the cingulum bundle, are altered following early maltreatment and these changes might relate to the structural covariance alterations observed here. However, the links between functional, white matter and grey matter structural connectivity during development remain poorly understood and more prospective multi-modal longitudinal studies are needed to test for links between structural, functional, and structural covariance alterations in the same regions.

6.4.4 Strengths and limitations

This chapter had several strengths. It was the first study to examine structural covariance in a group of individuals who experienced early institutional deprivation. Moreover, it was the first study that examined the effects of early maltreatment on structural covariance of both cortical thickness and surface area. We have shown that structural covariance provides a useful approach to study brain network alterations following maltreatment rather than isolated changes in brain morphometry. These changes might indicate alterations in maturational coupling in
early development and therefore offer the potential to investigate developmental processes in cross-sectional brain imaging designs such as this one.

There were several limitations to this chapter. The group comparison approach, which did not allow continuous factors to be investigated, limited us in two ways: First, we were not able to test the influence of deprivation duration as a continuous factor but relied on splitting the Romanian adoptees group into limited and extended deprivation exposure groups. We were therefore not able to test for a linear dose-dependent relationship between deprivation duration and structural covariance. Using the group comparison approach, there was only one significant difference between UK and Rom<6 with regard to surface area, while most other differences were found in Rom>6, suggesting that extended deprivation is associated with more widespread or stronger alterations in structural covariance. Second, we were not able to examine the influence of deprivation-related neurodevelopmental problems, such as increased symptoms of ADHD or ASD, on a continuous level. An alternative approach would have been to examine individuals with extended deprivation who meet diagnostic criteria for one of these disorders compared to those who do not, however, these comparisons would have been not powered enough in terms of sample size. In future studies, it would be interesting to compare four different groups in terms of structural covariance: individuals with and without early maltreatment exposure who either meet or do not meet the diagnostic criteria for a neurodevelopmental disorder.

6.4.5 Conclusion

In conclusion, this is the first study to show that institutional deprivation was associated with altered structural covariance indicating profound and long-lasting alterations in coordinated brain network development and maturational coupling.
Correlations between right fronto-temporal regions appeared to be most affected and cortical thickness and surface area networks showed distinct alterations, stressing distinct plasticity and vulnerability of both networks to early adverse experiences. Deprivation-related changes in fronto-temporal structural covariance might constitute vulnerability for subsequent psychopathology such as ASD or ADHD and it is likely that changes in structural covariance relate to alterations in functional and white matter connectivity.
CHAPTER 7

GENERAL DISCUSSION
7.1 HIGHLIGHTS

- This thesis aimed to study the impact of early childhood institutional deprivation on adult brain structure and structural covariance.
- We have shown that – more than 20 years after it has ended – institutional deprivation was associated with smaller total brain volume and regional cortical alterations while no effect was found for the volume of subcortical structures.
- These alterations were consistent with a model in which deprivation has a diverse range of effects on brain development, leading in some cases to manifest symptoms of disorder, in others latent vulnerability (marked by underlying cognitive weaknesses but no symptoms) and in others potentially neural compensatory effects associated with resilience.
- There was also preliminary evidence that ADHD and ASD symptoms expressed by those who experienced high levels of deprivation may have a different neural signature from those exposed to no or low levels of deprivation.
- We obtained evidence that deprivation also had long-term effects on the coordinated development of brain networks as indicated by structural covariance analyses of cortical thickness and surface area.
- This thesis had many strengths, such as building on a natural experimental design that overcomes many of the problems of previous studies of maltreatment and early adversity, but also limitations which should be considered, such as the cross-sectional nature of the imaging assessments.
- This study is the first to characterise the long-term impact of institutionalisation on brain structure in adulthood.
- Prospective longitudinal brain imaging studies are needed to investigate how early adverse experiences alter developmental trajectories to predict later brain development and well-being.
7.2 **OVERVIEW**

This chapter will first revisit the aims of this thesis and then summarise and discuss the findings of each of the empirical chapters in this context. Alternative group comparison approaches will be discussed followed by the strengths and limitations of the studies included in this thesis. Finally, I will discuss implications of the findings and future directions for research in this area.
7.3 AIMS OF THIS THESIS REVISITED

The infant human brain has a heightened ability to change in response to the environment in comparison to the plasticity seen in the adult brain (Ismail et al., 2017). This enables learning and healthy development on the one hand, but leaves the brain vulnerable to adverse environments, on the other (Sonuga-Barke, 2017). The long-term adverse effects of early maltreatment on psychopathology and well-being (Gilbert et al., 2009) indicate a strong neurobiological programming effect of early life events on brain development (Rutter & O’Connor, 2004). This hypothesis is supported by animal studies on early life stress (Lupien et al., 2009) and observational human studies on early maltreatment (McCroy et al., 2017; Teicher & Samson, 2016). However, most observational studies are limited in their ability to draw causal inferences due to the potential impact of familial genetic and environmental confounding (Sonuga-Barke et al., 2017). The English and Romanian Adoptees (ERA) Study overcomes some of these limitations by utilising a natural experimental design: the ERA is a longitudinal prospective study investigating a group of individuals who experienced profound and time-limited institutional deprivation followed by adoption into families that mostly provided stable and caring environments (Rutter et al., 2012). Previous ERA reports have shown that extended (>6 months) early institutional deprivation was associated with a persisting and distinct pattern of neurodevelopmental problems (Sonuga-Barke et al., 2017). Very little was known until now about the underlying brain mechanisms (Mehta et al., 2009).

As part of the ERA Brain Imaging Study (ERABIS), the aim of this thesis was to examine the effects of profound early childhood institutional deprivation on adult brain structure and structural covariance. Chapter 3 aimed to establish if institutional deprivation was associated with long-term alterations in cortical thickness, surface...
area and volume or subcortical volume. We also tested for deprivation duration effects on these measures within the Romanian adoptees. In Chapter 4, we explored whether these alterations were associated with neurodevelopmental and neuropsychological outcomes to test whether they represented potential compensatory effects, manifest disorder risk or latent cognitive vulnerability to disorder. In Chapter 5, we performed a whole-brain analysis across the cortex to test whether the association between brain structure and neurodevelopmental problems following extended deprivation showed a distinct pattern compared to the relationships seen following no or low deprivation. Finally, Chapter 6 explored whether early institutional deprivation was associated with alterations in coordinated brain network development as indicated by structural covariance of cortical thickness and surface area.
7.4 SUMMARY AND DISCUSSION OF MAIN FINDINGS

Early childhood institutional deprivation has a long-lasting impact on brain structure with smaller total brain volumes and regional cortical alterations observed in young adulthood. No deprivation-related effects were found for subcortical volumes.

Chapter 3 showed that not only was institutional deprivation associated with substantially smaller total brain volumes, but there was also a negative linear relationship between deprivation duration and total brain volume. This global effect could not be attributed to pre-existing genetic or prenatal risk nor subnutrition experienced during institutionalisation, emphasising the specific role of the negative effects of psychosocial deprivation on total brain volume. These findings are in line with the Bucharest Early Intervention Project (BEIP) that reported smaller brain volumes following institutional deprivation in childhood (Sheridan et al., 2012). This thesis showed that deprivation effects persist up until adulthood – more than 20 years after exposure has ended - despite the adoptees experiencing high-quality care in the interim period.

Beyond these global effects, there were more subtle regional alterations in brain structure. Cortical surface area, thickness and volume of a cluster including the right inferior temporal cortex were relatively larger in Romanian adoptees (Rom) compared to non-deprived UK adoptees (UK). In contrast, surface area and volume of a cluster in the right inferior frontal cortex were relatively smaller following institutionalisation. Surface area and volume of a cluster in the right medial prefrontal cortex, including the anterior cingulate cortex and medial orbitofrontal cortex, showed a positive relationship with deprivation duration in Rom. These findings partly supported our hypothesis that strongest alterations would be found in
the prefrontal cortex, consistent with previous studies on early maltreatment (Bick & Nelson, 2016; Hart & Rubia, 2012; McCrory et al., 2010; Teicher & Samson, 2016). However, it was surprising that some regions seemed to be relatively less affected compared to reductions in total brain volume, namely the inferior temporal and medial prefrontal cortices. This could have been caused by either relatively stronger growth in early childhood or reduced cortical thinning in later childhood (Gilmore et al., 2018).

Contrary to our hypotheses, subcortical volumes were not significantly associated with institutional deprivation nor were they related to neurodevelopmental problems. These findings stand in contrast with previous studies that show an effect of early maltreatment on amygdala and hippocampal volume (Teicher & Samson, 2016), even though findings have been mixed with regard to institutional deprivation (Bick & Nelson, 2016). One explanation of the discrepancy in findings might be that initial effects of deprivation on these structures disappear in adulthood (Teicher & Samson, 2016). While this would be consistent with our own pilot study, when the participants were aged around 15 years (Mehta et al., 2009), no institutional deprivation-related effects on either structure were found in childhood in the BEIP (Sheridan et al., 2012). Another possibility might be that different forms of maltreatment have a distinct impact on these structures (Cassiers et al., 2018).

It should be noted that all analyses of cortical surface area and volume and subcortical volumes controlled for total brain volume as a covariate. This means cortical and subcortical regions that did not show significant differences in this study, showed volume reductions that were proportional to the overall (on average 9%) smaller total brain volumes following institutionalisation and deprivation duration.
Deprivation appeared to have a diverse range of effects on brain development leading in some cases to manifest symptoms of disorder, in others to latent vulnerability (marked by neuropsychological impairments but no symptoms) and in others to neural compensations associated with resilience.

In Chapter 4, we started to test for associations between brain structure alterations and clinical or neuropsychological outcomes. In some cases, early deprivation-related changes appear to create risk for manifest symptoms of disorder. The global deprivation-related effect of smaller total brain volumes was associated with higher disinhibited social engagement (DSE) symptoms and lower IQ (Chapter 4), providing a potential link between early exposure and long-term neurodevelopmental outcomes. In other cases, they seemed to be related to latent vulnerability, marked by deficits in cognition but no associations with current disorder. The main example of this was the finding that the volume of the right medial prefrontal cluster was negatively associated with empathic accuracy in the group of adoptees with extended deprivation (Rom>6). This indicates – consistent with the latent vulnerability model (McCrory & Viding, 2015) – that deprivation duration-related increases in medial prefrontal volume are associated with impaired neuropsychological performance – which may reflect risk for future disorder. This may be relevant with regard to callous-unemotional traits, which are marked by a lack of empathy, and which were significantly higher in Rom>6 who had elevated symptoms of either DSE (Kennedy et al., 2017) or attention-deficit/hyperactivity disorder (ADHD; Kennedy et al., 2016).

There was also evidence of neural compensation where deprivation-related regional changes were associated with comparatively better outcomes, either fully manifest as fewer symptoms of disorders or associated with better neuropsychological performance - though not affecting symptoms (i.e., latent resilience). The possibility
that some brain structural alterations following early maltreatment might be the product of compensatory processes rather than impairment has been suggested previously (Teicher et al., 2016). We provide some of the strongest evidence to date for this hypothesis. First, deprivation-related effects on the volume of the right inferior temporal gyrus were associated with better prospective memory and fewer symptoms of ADHD in Rom>6. Deficits in prospective memory have been identified in individuals with idiopathic (non-deprivation-related) ADHD (Altgassen, Kretschmer, & Kliegel, 2014; Fuermaier et al., 2013) and ADHD symptoms mediated the relationship between deprivation and prospective memory in our sample (Golm et al., under submission). The underlying brain structural mechanism for this mediational effect might be changes in relative inferior temporal volume. Second, deprivation-related effects in the right inferior frontal cluster were associated with better proactive inhibition in Rom>6. It is plausible that compensatory processes affecting prospective memory and proactive inhibition might have a brain structural basis in these brain regions, as they have previously been linked functionally in normative samples (Cona et al., 2015; van Belle et al., 2014). We can only speculate on potential mechanisms that facilitate these compensatory processes and why they are seen in some individuals but not others. One potential factor (among others, such as genetic markers and specific exposures during institutionalisation) could be the post-adoptive environment. It is plausible that certain influences post-adoption, which was mostly marked by an enriched, nurturing and supportive environment, might have led to later plastic changes in brain-behaviour links promoting resilience.
There was initial evidence that neurodevelopmental symptoms that arise following extended deprivation have a distinct brain structural signature.

The findings above indicate that institutional deprivation potentially alters the pathways between brain structure, cognition and neurodevelopmental outcomes. This was further investigated in Chapter 5 using a whole-brain approach and testing for interactions between deprivation status and neurodevelopmental symptoms. We found that neurodevelopmental symptoms in Rom>6 had a distinct relationship with brain structure compared to the association seen in the group of individuals with no or limited deprivation (LoDep). ADHD symptoms were positively associated with gyrification in bilateral supramarginal, right lingual and right superior frontal cortex in Rom>6 only. These relationships were non-significant in LoDep and have also not been observed in studies investigating patients with ADHD (Ambrosino et al., 2017; Forde et al., 2017; Shaw et al., 2012). This suggests that excessive folding of these brain regions might be a potential mediator for a form of ADHD that is associated with extended deprivation and which has also shown other uncommon characteristics: It showed high persistence into adulthood, similar rates of males and females, was mostly of the inattentive type and had little comorbidity with conduct disorder (Kennedy et al., 2016). A similar interaction between deprivation status and symptoms was found with regard to autism spectrum disorder (ASD), a condition that is thought to be largely genetically determined (Tick et al., 2016), while persistently increased symptoms in Rom>6 support the existence of a form of ASD with a largely environmental cause. In Rom>6 only, ASD symptoms were positively associated with surface area in the left superior parietal cortex. This association was not found in LoDep nor was it reported in studies investigating patients with ASD (e.g. van Rooij et al., 2017), suggesting a potential deprivation-specific brain structural marker for ASD. Deprivation status also moderated the
relationship between IQ and brain structure. In Rom>6, IQ was positively associated with surface area and volume in the left lingual cortex but negatively with thickness in the left rostral middle frontal and volume in the right superior parietal cortex. In contrast, these associations were non-significant or reversed in the LoDep group, in line with studies in the general population that mostly find positive associations between IQ and fronto-parietal brain structure (Basten et al., 2015; Deary et al., 2010; Jung & Haier, 2007). Cognitive impairment showed substantial recovery up until young adulthood in ERA, while it is thought to be relatively stable in the general population from age 7 onwards (Schneider et al., 2014). These findings might indicate a possible neuroplastic restructuring that allowed for such recovery.

Together, these brain structural alterations might mediate the distinct characteristics associated with deprivation-related ADHD, ASD and cognitive impairment as described in previous reports of this sample. These findings form initial evidence that deprivation-related neurodevelopmental disorders might be distinct from their idiopathic variants in brain structural underpinnings as suggested by Teicher and Samson (2013).

Institutional deprivation appeared to also affect the organisation of brain networks – with altered correlations of cortical thickness and surface area between affected brain regions.

We found evidence that deprivation was associated with altered patterns of structural covariance in young adulthood, suggesting early effects on the organisation of brain networks (Chapter 6; Alexander-Bloch, Raznahan, et al., 2013). These deprivation-related differences in correlational strength and/or direction mostly implicated brain areas identified as showing structural effects in Chapter 3 - with fronto-temporal connections in the right hemisphere mainly being
affected. While we found overlap in deprivation-related regional alterations in cortical thickness and surface area in Chapter 3, the two measures were largely distinct with regard to structural covariance alterations. This was in accord with previous studies (Sanabria-Diaz et al., 2010; Schmitt et al., 2018; Wierenga et al., 2014). Altered structural covariance of cortical thickness has previously been associated with early maltreatment (Sun et al., 2018; Teicher et al., 2014) and neurodevelopmental disorders such as ASD and ADHD (Bernhardt et al., 2014; Bethlehem et al., 2017). These findings suggest that early deprivation is associated with alterations in coordinated brain development that persist as changes in structural covariance up until adulthood. These changes might represent manifestations of early differences in functional connectivity (Geng et al., 2017) and/or white matter organisation (Moura et al., 2017), highlighting potential pathways between early life experiences and associated psychological outcomes and psychopathology more than two decades later.

Together, these findings show that institutional deprivation has a strong neurobiological programming effect on brain development highlighting how environmental influences during periods of heightened neuroplasticity can lead to persistent changes in brain structure.

This thesis is the first to show that deprivation during a precisely-timed period of the first months of life is associated with changes in adult brain structure. These changes partly explained neurodevelopmental and neuropsychological outcomes. This study therefore supports theories of sensitive periods of heightened plasticity early in life, during which environmental influences can have a strong neurobiological programming effect that is associated with brain structural changes more than 20 years after exposure to the environment has ended (Rutter &
O’Connor, 2004). All theories of sensitive periods of neuroplasticity propose that early adverse experiences during these periods can have persistent effects on brain development with only limited subsequent plasticity in response to changes in later environment (Marshall & Kenney, 2009). Some of these theories propose higher risk for psychopathology following early adversity might arise from brain structural and functional adaptation to early adverse environments (which is maladaptive in later benign environments), such as experience-adaptive programming (Rutter & O’Connor, 2004) and latent cognitive vulnerability (McCrory & Viding, 2015). In contrast, models of experience-expectant programming propose that healthy development requires certain stimulation during sensitive periods and exposure to non-normative environments that lack such stimulation will have permanent negative effects on development (Greenough, Black, & Wallace, 1987). While models of experience-adaptive programming consider individual differences in response to adversity, experience-expectant programming should be associated with the same adverse effects in all individuals (Marshall & Kenney, 2009). Overall, findings of this thesis support a model, where brain structural changes are associated with adaptation and maladaptation that partly explain a deprivation-specific neurodevelopmental pattern that is present in many of the adoptees exposed to deprivation but also shows high intra- and inter-individual variability. We interpret this as evidence for experience-adaptive programming and latent cognitive vulnerability, even though it cannot be ruled out that some of the effects might show characteristics of experience-expectant programming.

A graphical illustration of the main findings of this thesis can be found in Figure 7.1.
Figure 7.1: Main findings of this thesis.
7.5 GROUP COMPARISON RATIONALE REVISITED

As introduced in Chapter 2, different approaches to configure institutional deprivation as an independent variable were required across chapters to address different research questions. Where possible, deprivation duration was used as a continuous variable (Chapter 3) but some analyses required deprivation duration to be treated as a categorical predictor (> 6 months versus LoDep) either because of statistical power considerations (Chapters 4 and 5) or because the analyses only allowed group comparisons (Chapter 6). I will now review the implementation of these approaches, potential limitations and alternative approaches that we considered.

7.5.1 Chapter 3

For Chapter 3, UK and Rom groups were compared to investigate the effect of institutionalisation per se. We subsequently performed a linear regression with deprivation duration within the Rom group to test for a dose-dependent relationship. This approach presented the fewest assumptions on the relationship between deprivation and brain structure. An alternative approach might have been to compare adoptees with no deprivation (UK group), limited deprivation (Rom<6) and extended deprivation (Rom>6) or even combining the no and limited deprivation groups (LoDep). However, testing for a continuous deprivation duration relationship was considered the most powerful alternative in any model where brain structure did not show a clear step-wise difference at 6 months of deprivation duration. A limitation of this approach is that it did not formally test for step-wise differences in brain structure at the 6 months deprivation duration threshold. However, this threshold would be more informative in conjunction with the analysis of neurodevelopmental symptoms, which was not the focus of this chapter.
7.5.2 Chapters 4 and 5

Chapters 4 and 5 aimed to investigate the relationship between brain structural alterations and neurodevelopmental symptoms. LoDep and Rom>6 groups were compared to examine the relationship between deprivation status, brain structure and neurodevelopmental/ neuropsychological outcomes. As stated in 2.7, 6 months was chosen as a cut-off for limited vs extended deprivation, as this threshold has been used in previous reports of ERA and was consistently associated with a step-wise increase in neurodevelopmental symptoms (Sonuga-Barke et al., 2017). Low symptom counts within the UK adoptee group (and the small size of this group) prohibited alternative approaches such as comparing UK and Rom groups. It would have been possible to use deprivation duration as a continuous variable within Rom alone. However, this approach would not have allowed inferences on the relationship between brain structure and neurodevelopmental outcomes in a group of non-deprived individuals. Therefore, comparing LoDep and Rom>6 was considered the most informative model to answer the research questions of Chapters 4 and 5.

7.5.3 Chapter 6

As this was the first study to investigate the impact of institutional deprivation on structural covariance, we were interested to examine the effects of institutionalisation per se and deprivation duration, similar to the approach in Chapter 3. However, the structural covariance analysis relied on group-level inferences and was consequently limited to categorical group comparisons that did not allow to examine the effects of continuous factors such as deprivation duration and symptom levels. In order to investigate if deprivation duration altered structural covariance, this analysis was therefore reliant on choosing categorical groups.
representing limited and extended deprivation. A 6 months cut-off was in line with previous analyses in this thesis and previous ERA reports. However, this analysis did not relate directly to neurodevelopmental symptoms. We addressed the criticism that 6 months might not be the most sensitive threshold by performing sensitivity analyses using different thresholds. These provided support for the 6 months threshold.

Finally, it should be noted that deprivation duration varied between 3 and 41 months in this sample and only relatively few participants were close to the 6 months cut-off. Changing the threshold to 8 months, for instance, would only change the status of 2 participants. On average, deprivation duration in the extended deprivation group was 16 months. Effects of extended deprivation should be interpreted in this light.
7.6 STRENGTHS

This thesis had several strengths. First, this was the first study to investigate the impact of institutional deprivation on brain structure and covariance in young adulthood, more than 20 years after exposure has ended. While observational studies exist that link early maltreatment to adult brain structure (Teicher & Samson, 2016), these often had the limitation of continued adversity: adverse environments in early life were likely to remain adverse throughout development. This study shows a strong and long-lasting neurobiological programming effect of precisely timed deprivation in early childhood with stronger effects following extended deprivation.

Second, due to its natural experimental design and the availability of a comprehensive set of measures including general growth, subnutrition and birth weight, this study strengthens one’s ability to draw inferences about the causal role of adverse environmental exposures in brain development, providing some of the strongest evidence to date that institutional deprivation alters brain structure. The design of this study overcomes many of the limitations associated with retrospective observational designs, such as familial confounding and limited validity of retrospective reports and confounding of maltreatment and psychopathology due to the use of clinically-referred samples. It therefore seems unlikely that the effects reported in this thesis can be attributed to other pre-existing factors such as genetic risk or prenatal environment or later adoptive environment.

Third, the fact that extensive clinical and neuropsychological assessments of the participants were performed allowed us to characterise brain-behaviour or brain-psychopathology relationships and investigate the functional consequences of changes in regional brain structure. Beyond manifest disorder risk and latent cognitive vulnerability, we were able to show that some deprivation-related changes
in brain structure were likely the result of neural compensation either expressed fully
as resilience from disorders symptoms or as latent resilience in the form of better
neuropsychological outcomes without less symptoms. This is in line with findings
that show structural brain alterations in individuals with early maltreatment but
without psychopathology (Teicher et al., 2016) but has rarely been tested directly.

Fourth, linking brain structural changes to neurodevelopmental and
neuropsychological outcomes was facilitated and strengthened by the availability of
longitudinal data and reports to characterise this sample. For example, previous
reports have established and documented the neurodevelopmental pattern of
problems associated with extended deprivation (comprising increased symptoms of
ADHD, ASD and DSE and cognitive impairment (as indexed by IQ)). It stands to
reason that brain structural changes might be related to these symptoms. We were
then able to use parent-rated symptom counts as collected by the previous follow-
up and combine these with measures collected during ERABIS.

Fifth, this was the first study to investigate the effects of early institutional
depprivation on structural covariance and the first study on childhood maltreatment
that investigates structural covariance of both cortical thickness and surface area.
Not only were we able to show that institutional deprivation was associated with
alterations in the coordinated development of brain regions, but we also showed
that the effects on structural covariance were distinct for cortical thickness and
surface area, stressing the importance of considering both measures.

Six, most other studies on the effects of early institutionalisation included a
comparison group of non-adopted controls. A comparison group of individuals who
were also adopted but did not experience any institutional deprivation allowed to
control for the effects of adoption per se as well as other factors such as above-
average income adoptive homes (Rutter, 1998).
General discussion

Taken together, this thesis provides the first comprehensive exploration of long-term changes in brain structure following early institutional deprivation. To our knowledge, it is also the first to characterise links between such brain structural changes and neuropsychological performance and clinical symptoms.
7.7 **LIMITATIONS**

This thesis also had several limitations which should be considered.

First, because of the limited sample size, we were only adequately powered to detect medium or large effects. Even though not directly comparable to this study, a previous study on statistical power in FreeSurfer analyses suggests that sensitivity to detect group differences varies across the cortex and across measures, with cortical surface area generally being more sensitive (Liem et al., 2015). Subtler differences in brain structure might not have been identified in this study. The small sample size was a result of relatively high attrition in ERABIS as discussed in 2.8. While there was no strong indication for selective attrition overall, Rom>6 with higher rates of ASD symptoms at age 6 were more likely to have dropped out. Therefore, inferences on the relationship between deprivation, brain structure and ASD symptoms might be limited in this study.

Second, even though ERA is a longitudinal study, the brain imaging study was cross-sectional. This means that our conclusions on alterations in brain development can only be based on one structural MRI scan acquired in young adulthood. The sample of six participants who participated in both the ERABIS pilot study during adolescence and this follow-up was not big enough to allow a longitudinal investigation. We were therefore not able to draw inferences regarding altered developmental *trajectories*, which have been studied in neurodevelopmental disorders such as ADHD (Ambrosino et al., 2017) and ASD (Zabihi et al., 2018). There is growing recognition that the effects of early maltreatment might affect such trajectories (Teicher & Samson, 2016) although only a handful of longitudinal studies of maltreatment exist (e.g. Whittle et al., 2013). For example, while we did not find significant differences in subcortical volumes such as amygdala and hippocampus following early institutional deprivation, we cannot rule out that volume...
alterations might have been present in childhood or adolescence, as suggested by our pilot study and other studies which investigated the effects of early maltreatment on subcortical volumes.

Third, while ERA has collected a wealth of neuropsychological data via assessments and interviews throughout development, only a subset of this information could be considered for this thesis. For example, when linking brain structure to neurodevelopmental outcomes, we decided to focus on the four domains previously identified as sensitive to deprivation (Kumsta, Kreppner, et al., 2010). However, the latest follow-up has shown a young adult onset of increased symptoms of mood and anxiety disorders (depression and general anxiety disorder) in Rom>6 (Sonuga-Barke et al., 2017). Brain structural abnormalities have been reported in patients with mood disorders and some of the key areas implicated in this thesis, such as the anterior cingulate, medial orbitofrontal and inferior temporal cortices, have also been shown to be altered in adults with depression (Schmaal et al., 2016). In our study, anterior cingulate volume was negatively related to empathic accuracy but not neurodevelopmental symptoms in Rom>6. Consistent with the theory of latent cognitive vulnerability (McCrary & Viding, 2015), one could hypothesise that this vulnerability might constitute a risk factor for the development of mood disorders and this should be investigated in further studies.

Fourth, related to the points above, this thesis focused on neurodevelopmental and neuropsychological outcomes acquired in young adulthood. It would be interesting to link brain structure to developmental trajectories. For example, while elevated symptoms of ADHD, ASD and DSE showed high persistence throughout development, cognitive impairment showed substantial recovery while mood disorder symptoms showed a striking increase in late adolescence/early adulthood (Kennedy et al., 2017; Kennedy et al., 2016; Sonuga-Barke et al., 2017). It would be
fascinating to study how brain structure differs in individuals who would be classified as resilient, recovering or persistent in either of these domains.

Fifth, it should be noted that we were only able to investigate neurodevelopmental disorders such as ADHD, ASD and DSE on a symptom rather than diagnosis level and that symptoms of ADHD and ASD were assessed with parent-rated questionnaires rather than clinical interviews. By not assuming a clinical diagnosis as a categorical cut-off, we were able to investigate symptoms as a continuum, but we cannot make inferences on clinical disorders per se and comparability with studies that compare groups of clinically diagnosed patients might be limited. In this light, Chapters 4 and 5 should be considered as providing initial evidence that deprivation-related neurodevelopmental disorders might be distinct from non-deprivation-related, idiopathic forms of these disorders, while this should be formally tested by comparing Romanian adoptees who meet criteria for clinical diagnoses with a group of non-deprived patients with the same diagnosis.

Sixth, while the UK comparison group was chosen carefully as an adopted group that did not experience institutional deprivation, there are also certain limitations: First, UK and Romanian adoptees might show differences based on ethnicity. However, ethnic differences are unlikely to be the cause of the large effect sizes found in this study. Second, neurodevelopmental symptom rates in the UK group were relatively low and it would be interesting to recruit additional groups of patients with higher symptom rates as comparison groups.

Seventh, as most adoptees entered the institutions right after birth, we were unable to disentangle the effects of deprivation duration from the timing of the start of the deprivation exposure. Therefore, we were unable to draw inferences regarding sensitive periods of different brain structures and functions other than highlighting the importance of the first years of life more generally.
7.8 IMPLICATIONS

An estimated 8 million children live in institutions around the world (Dunn, Jareg, & Webb, 2007). The overwhelming majority of these children (at least 80%) are not orphans but have one or both parents alive (Csáky, 2009). Extreme poverty – often compounded by other circumstances such as social discrimination or disability – is one of the main reasons why families may see it as their only option to place their children into institutional care (Better Care Network, 2009). While the quality of institutional care varies and might not always be as appalling as found in Romanian institutions under the Ceaușescu regime, they cannot replace functioning family structures (Lumos Foundation, 2017). This view is supported by a large body of research showing the negative developmental impact of institutionalisation which align with the findings of ERA (Berens & Nelson, 2015).

In this thesis, we have shown that institutional deprivation has a long-term negative impact on brain development with effects still seen in young adulthood – more than 20 years after children were removed from the institutions, and despite the fact that the children received high quality care in the interim. While some of these changes in brain structure appear compensatory, others such as smaller total brain volume appeared to increase risk for disorder or symptoms. We have presented strong evidence that the effect of institutional deprivation on brain structure is causal. Importantly, brain structural alterations reported here were independent of subnutrition as indexed by weight at UK entry. This finding stresses the impact of the psychosocial aspects of deprivation and is further supported by other studies that show that attachment and social behaviour are profoundly impacted by institutional deprivation (Kennedy et al., 2017; Olsavsky et al., 2013; Sonuga-Barke et al., 2017). Replacing institutional care with models of family-based
and community-based care may minimise the effects on early development, and have started to be successfully implemented in some countries (Gîrlescu, 2018).

The knowledge gained through this work could lead to potential new pathways of intervention and prevention of the negative impact on neurodevelopment following institutional deprivation. Importantly, not all brain structural alterations observed here were associated with increased disorder risk or vulnerability – instead they appeared to be linked to successful compensation in some cases (fewer ADHD symptoms or better prospective memory performance). Harnessing and targeting these compensatory changes in brain development could prevent onset of psychopathology. For example, better prospective memory was associated with lower ADHD symptoms in Rom>6 (possibly mediated by inferior temporal volume). Prospective memory could be targeted specifically by training in strategic time monitoring (Geurten, Lejeune, & Meulemans, 2016), which could be incorporated in a wider programme of intervention and prevention. However, longitudinal intervention studies are needed to show that the intervention leads to a normalisation of brain structure over time, and in parallel, improvements in neuropsychological performance or reductions in symptoms.

Another approach would be to target brain structural changes and neurocognitive functions that might reflect latent cognitive vulnerability (McCrory & Viding, 2015). Identifying individuals who present this vulnerability before the onset of symptoms will be crucial for the successful implementation of interventions that might prevent psychopathology before it arises. For example, we have shown that empathic accuracy was impaired in Rom>6 and this was associated with larger right medial prefrontal volumes. Impaired ability to empathise with others increases risk of certain types of psychopathology (Kim & Cicchetti, 2010). Interventions could be implemented that target empathic ability and empathic motivation directly (Weisz &
Zaki, 2018) and this might be more successful than trying to treat the resulting symptoms only.

Finally, we presented initial evidence that deprivation-related forms of neurodevelopmental problems might have distinct brain structural underpinnings from their non-deprivation-related counterparts. This is supported by findings that show that deprivation-related psychopathology is distinct in other respects such as higher persistence and comorbidity (Teicher & Samson, 2013). Together, these findings suggest that neurodevelopmental problems caused by early maltreatment or institutionalisation might need to be treated differently from their idiopathic variants and therefore early maltreatment experiences should be assessed and considered in future studies investigating treatment efficacy.
7.9 **FUTURE DIRECTIONS**

Some important questions for future research will be: How does early maltreatment and specifically early institutional deprivation alter brain structural *trajectories* to *predict* neurodevelopmental and neuropsychological outcomes and where are the potential avenues for *intervention and prevention*?

To answer these questions, longitudinal prospective and multimodal brain imaging studies are needed which eventually incorporate intervention designs. To date, research utilising longitudinal brain imaging methods to investigate the impact of early maltreatment on developmental trajectories is in its infancy. These studies are needed to form a better understanding of how alterations in brain structural trajectories map on neurodevelopmental trajectories. Multimodal imaging will help to investigate how grey and white matter structural and functional brain alterations map onto each other. These studies will also provide a better understanding of markers of risk and resilience underlying the inter- and intra-individual heterogeneity in maltreatment-related developmental outcomes.

The next years will also see increased efforts to identify early biomarkers that can predict later neurodevelopmental disorders such as ASD and the most beneficial treatments on an individual basis (Loth et al., 2017). Such studies will benefit from incorporating assessments of early maltreatment in their designs as our data suggest that it might be associated with a distinct presentation of the disorder and potential differences in the type of treatment that might be needed.

Natural experimental designs such as ERA provide the unique opportunity to disentangle the impact of early maltreatment from familial genetic and environmental influences. Further research using this study design will provide invaluable insights into the causal effects of deprivation throughout the lifespan. It is important to also study the effects of other forms of maltreatment such as abuse
and neglect experienced within the family. Observational studies in this field have started to assess genetic risk scores (Croft et al., 2019), which have become more predictive (Krapohl et al., 2017). If applied within a prospective study design that includes comprehensive measures of maltreatment exposure and neurodevelopment, this will strengthen inferences on the developmental effects of early maltreatment and its interplay with genetic risk and resilience factors.
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APPENDIX
Appendix A: Items used for each symptom domain at young adulthood.

18 DSM-5 ADHD symptoms were measured with 20 parent-rated items of the Conners Comprehensive Behaviour Rating Scales. ASD symptoms were assessed with 15 items of the parent-rated Social Communication Questionnaire. DSE symptoms were assessed with 3 interview questions.

<table>
<thead>
<tr>
<th>ADHD</th>
<th>Hyperactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inattention</strong></td>
<td><strong>Hyperactivity</strong></td>
</tr>
<tr>
<td>1. Forgetfulness (1 item)</td>
<td>1. Cannot wait to answer (1 item)</td>
</tr>
<tr>
<td>2. Makes careless mistakes (1 item)</td>
<td>2. Cannot stay seated (1 item)</td>
</tr>
<tr>
<td>3. Lack of organization (1 item)</td>
<td>3. Restlessness (1 item)</td>
</tr>
<tr>
<td>4. Avoidance of tasks that require sustained effort (1 item)</td>
<td>4. Cannot wait for their turn (1 item)</td>
</tr>
<tr>
<td>5. Unable to listen (1 item)</td>
<td>5. Talking too much (1 item)</td>
</tr>
<tr>
<td>6. Loosing things (1 item)</td>
<td>6. Fidgety (1 item)</td>
</tr>
<tr>
<td>7. Cannot sustain attention (1 item)</td>
<td>7. Noisiness (1 item)</td>
</tr>
<tr>
<td>8. Easily distracted (1 item)</td>
<td>8. Interruption (1 item)</td>
</tr>
<tr>
<td>9. Unable to complete tasks (two items)</td>
<td>9. Constant movement (at least one of two items)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Autism spectrum Disorder</th>
<th>Repetitive and Stereotyped Behaviors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Communication</strong></td>
<td><strong>Social Reciprocal Interaction</strong></td>
</tr>
<tr>
<td>10. To and fro conversation</td>
<td>21. Attempts to comfort</td>
</tr>
<tr>
<td>11. Socially appropriate</td>
<td>23. Normal range of facial expressions</td>
</tr>
<tr>
<td>12. Difficulties with pronouns</td>
<td>24. Appropriate facial expressions</td>
</tr>
<tr>
<td>13. Uses made up words/ phrases</td>
<td>28. Responds positively to others</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disinhibited Social Engagement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Seemed to friendly with strangers or too eager to approach strangers?</td>
</tr>
<tr>
<td>2. Made very personal comments or asked intrusive questions of others they’ve just met?</td>
</tr>
<tr>
<td>3. Seemed aware of social boundaries or the closeness of interaction with whom they are not familiar?</td>
</tr>
</tbody>
</table>
Appendix B: Standardized instructions for fMRI and neuropsychological tasks performed during ERABIS.
# ERABIS – Instructions for training exercises

**fmRI TASKS**

<table>
<thead>
<tr>
<th>Task</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RESTING STATE</strong></td>
<td>Please keep your eyes open and keep looking at the cross during this scan.</td>
</tr>
<tr>
<td><strong>FACES TASK</strong></td>
<td>In this task you will see lots of people’s faces flashing onto the screen one at a time. All you need to do is identify whether the face is of a man or a woman. If you see a woman’s face push the left button as fast as you can, and if you see a man’s face push the right button as fast as you can. Don’t worry if you make a mistake, just carry on with the next face.</td>
</tr>
<tr>
<td><strong>STOP TASK</strong></td>
<td>In this task you will see an arrow on the screen pointing either left or right. Using the button box, all you need to do is press the button on the same side as the arrow is pointing. Most of the time you will just do this. But, sometimes you will see a dot appear on the screen just after the arrow, and this is either red or green. When you see the red dot appear, you should try to not press the button you were going to press. This is a difficult thing to do, and the task is actually designed to make it difficult for you – so you will probably make lots of mistakes. Don’t worry when you make a mistake, just keep going. When you see the green dot appear, you should ignore it and keep on pressing the button on the side the arrow is pointing.</td>
</tr>
<tr>
<td><strong>MID TASK</strong></td>
<td>This is a task that measures your reaction time. If you respond quickly you can win money. If you don’t respond quickly you could lose money. At the end of the task you will actually win a proportion of what you win in this task. For the task I’d like you to look at the screen and press the left button on the button box whenever you see a white square flash in the middle of the screen. Whenever the white square appears just press the button as fast as you can. On each go you’ll see a symbol before the white square. If it is a circle with lines inside it means that you will win points if you respond quickly. We’ve labelled the points as pounds so they are easy to understand. If you see this symbol and respond quickly to the white square you’ll win £2.00: (CIRCLE THREE LINES). If it is a square with lines inside it means that you will lose points if you don’t respond quickly. If you see this symbol and don’t respond quickly to the white square you will lose £2.00 (SQUARE THREE LINES). This symbol means you’re winnings won’t change but we’d still like you to respond as fast as you can: (TRIANGLE). Sometimes you’ll see this symbol: (CROSS). When you see this you don’t have to do anything. Just wait for the next symbol.</td>
</tr>
</tbody>
</table>
Appendix

ERABIS – Instructions for training exercises

Most people that have tried this task win about £15, but you could win over £25.

From here run presentation. Explain slides as you go.
This symbol means you could win £2.00, if you respond quickly to the white square.
This symbol means you could lose £2.00, if you don’t respond quickly to the white square.
You won’t win any money for this symbol, but still try and respond as quickly as you can to the white square.
And when this cross appears you don’t need to do anything – just wait for the next trial.

Set up participant in mock scanner.
Let’s have a practice of the MID task.

Run the practice playlist describing symbols and outcomes.
This symbol means you could win/lose…
We will be measuring your reaction times and will keep you informed of how much you have won. Just try and respond as fast as you can. Remember most people that have tried this task win about £15, but you could win over £25. Remember that you will be given a proportion of this at the end of the task.

5. EMPATHIC ACCURACY TASK

In this task you will be shown some short video clips of people talking about an event in their past. While watching we would like you to rate the strength of the emotion you think the person talking in the video clip felt when talking about this event. We do not want you to rate the emotion the person might have experienced during the event itself (e.g. The person might have been very scared when the event itself happened, but not when he/she was talking about it).

Open the ppt file “Instruction EAT” and change slides accordingly. Read instructions to participant.
This is the scale you are going to use to rate the videos. It runs from 1 (no emotion) to 9 (very strong emotion). The number that is marked orange will automatically be recorded. The scale is controlled by the two buttons on the button box. Press the right button to rate higher values on the scale and the left button for lower values. You will have the chance to practise in a moment.

Remember, you will be rating the strength of emotion you think the person is feeling while speaking in the video – not during the event itself.
You will be asked three questions after each video clip.

1) You will be asked to choose the emotion you think the person in the video felt the most while speaking (e.g. frightened, sad, happy, etc.).

2) Next, you will be asked to choose the emotion you felt the most while watching the video

3) Finally, you will be asked how strongly you felt that emotion.

Now we will practise. Do you have any questions?

Open file: ‘Example Video’ and rate clip explaining what you are doing.
Let them repeat the instructions in their own words and correct them if necessary.
Do you have any questions?

Open file: ‘Pre-training’ and allow participant to practice on the two practice video clips.
## NEUROPSYCHOLOGICAL TASKS

<table>
<thead>
<tr>
<th>Task</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EMOTION RECOGNITION TASK</strong></td>
<td>(standardised instructions – MM)</td>
</tr>
<tr>
<td><strong>REACTION TIME TASK</strong></td>
<td>In this task you will see two circles on the screen. One will be filled in yellow, the other will be just an outline (point to shapes on screen). All you need to do is press the left arrow key if the filled circle is on the left (demonstrate) – or press the right arrow key if the filled circle is on the right (demonstrate). Don’t worry if you make a mistake, just keep going with the next circle. There are two runs of this task.</td>
</tr>
<tr>
<td><strong>GO/NO-GO TASK</strong></td>
<td>This task measures your reaction time. You need to react by pressing the left button every time you see a CIRCLE. However, sometimes you will see a big CROSS, and when you see this you should not press the button. Before the shape appears on the screen, you will see either a small red cross, or a small white cross. When you see a red cross, this tells you that you will either see a CIRCLE next OR a big CROSS next. When you see the CIRCLE you need to press the button as quickly as possible. When you see a large CROSS, you need to not press the button. When you see a small white cross, this tells you that the next symbol you will see is a CIRCLE and that you will need to push the button as quickly as possible when you see this. Play the practice playlist and for the first few trials you play while describing what is happening, then instruct the participant to play.</td>
</tr>
<tr>
<td><strong>RISKY CHOICE TASK</strong></td>
<td>This task is called the Wheel of Fortune Game. You will win or lose points from ‘wheels’. Each wheel has 8 segments that are different colours. The number displayed in each segment tells you the amount you stand to win or lose; positive numbers are possible wins, and negative (minus) numbers are losses. The numbers of each colour tell you the chances of winning: for example if there are 4 blue segments and 4 red segments, there is an equal chance of winning or losing. <strong>Hit space to see example spin</strong> On each trial, we would like you to choose one of two wheels to play, using the ‘V’ and ‘B’ keys to select the left or right wheel. Let’s practice one of these. <strong>Press for a practice trial</strong> Sometimes, one wheel will offer a certain outcome: all the numbers will be the same. On these goes, you must choose whether to take the sure outcome or take a chance on the other wheel. Let’s practice one of these.</td>
</tr>
</tbody>
</table>
### ERABIS – Instructions for training exercises

<table>
<thead>
<tr>
<th>Press for a practice trial – after this trial, there is a delay of about 5s before it continues</th>
</tr>
</thead>
<tbody>
<tr>
<td>You will play several games. You start each game with 100 points, and you will play four games in total. The aim of each game is to win as many points as possible. Do you have any questions?</td>
</tr>
</tbody>
</table>

v3: SS 30.09.2014
Appendix C: Participant Information Sheet.
PART I

Purpose of the research

Brain imaging is currently used for a number of reasons including investigations into the structure and function of the brain as a result of age and experience. We are interested in understanding more about the relationship between different types of early experiences and the way in which the brain processes information in adulthood.

Do I have to take part?

Participation in the study is voluntary. It is not obligatory. We will describe the study to you, go through this information sheet, and provide a copy for you to keep. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving any reason. Some of the questions may touch sensitive topics. Of course none of the questionnaires or tasks are mandatory and you may choose to not answer certain questions or engage in certain parts of the study.

What is involved?

The study will involve visiting the Centre for Neuroimaging Sciences for two sessions amounting to a total of 8 hours in average. The two sessions (each of 4 hours in average) can take place on either one or two days, depending on what is convenient for you. The overall study will be on-going for approximately 3 years. We aim to have 322 volunteers complete the study.

Your visit

Each of the two sessions will be divided into two parts. One part will involve having a Magnetic Resonance Imaging (MRI) scan, and in the other part of the session you will be asked to complete some tasks on a computer and answer some questionnaires and have a short interview.

Questionnaires and interview:
In addition to the MRI scanning, we would also like you to fill in some questionnaires about your thoughts and feelings about everyday situations, and about your day to day behaviours. These can be completed using a computer before you come in for your appointment or during the day when you come in. When you visit we will administer a standard IQ test, a memory test and ask you some questions about your psychological health.

**DNA Sample:**
You will be asked to provide a mouth swab using a special brush to wipe the inside of your mouth. This is quick and easy to do and does not hurt at all. The samples will be used for research purposes only and do not constitute a genetic test of any sort. Therefore, we will not be giving the results of your individual genetic data to anyone. All genetic data will be stored anonymously and securely, and will be identified by code number only. The DNA will be stored until our research is completed and safely destroyed after that time. If you have been taking part in the Young Adult Follow-Up of the Family Research Project, we will probably not require another sample of your DNA.

**MRI Scanning**
The study will be using a magnetic field to help generate pictures of your brain, therefore you must not have a scan if you have received metal-associated injuries to your eyes, had metallic objects (including clips) inserted into your body during an operation, or if you have received a gun-shot injury or have a heart pace maker. The study team will go through a list of possible risks with you at screening as will the person conducting the scan before you go into the scanner. You will also be given the opportunity to lie in a mock scanner at the Centre for Neuroimaging Sciences before lying in the real scanner. This will help you become accustomed to the scanning environment.

**Computer tasks:**
If you take part in the study you will be asked to complete some simple tasks on a computer while you are in the scanner. There will be a practice session before your scan, in which you will receive instructions on how to complete the tasks. You will be asked to look at a computer screen and press a button in response to certain images. You will also be asked to complete some computer tasks outside of the scanner.

**What is MRI scanning?**
We use a very modern method of scanning known as Magnetic Resonance Imaging (MRI). This technique is commonly used to diagnose a number of diseases, but in this case it has also been adapted to take images of which parts of your brain are active when you are at rest or performing a task. When a part of your brain is more active, more blood flows to that region and this change is captured on the images that we take. We will make a map of which parts of your brain has more blood flow than others.

In order for us to take pictures of your brain, you will have to lie as still as possible in the MRI scanner. The scanner consists of a powerful magnet, but you will not feel any force or special sensation inside a magnetic field because your body is insensitive to it. Because of the magnetic field, you must not have a scan if you have received metal injuries to your eyes, had metallic objects (including clips) inserted into your body during an operation, or if you have received a gun-shot injury or have a heart pace maker. The radiographer will go through a list of possible risks with you before you go into the scanner. Please note that MRI scans do not involve any form of ionising radiation (X-rays), but the scanner itself can be quite claustrophobic; therefore please inform us if you have a fear of enclosed spaces.

All the time you are in the scanner there will be a microphone switched on so you can talk to us. We will talk to you regularly to explain what will happen next. Some people find the machine a little noisy, but the headphones we provide allow adequate noise protection for most people.
Before and after your scan
If you decide to take part in this research study we ask that before each study visit you:

- do not drink alcohol or engage in strenuous exercise (e.g. heavy lifting, aerobics) for 24 hours
- do not take products containing caffeine on the study day(s)
- abstain from nicotine- or tobacco- containing products for at least 4 hours before arrival at the Centre

During the study day we will provide food but we do ask you to abstain from nicotine-, tobacco- or caffeine- containing products until you leave the centre. We ask you to do this because these products can sometimes interfere with the blood flow measurements we take in the MRI scanner.

Will I benefit from my participation?
We do not expect that you will draw any specific personal benefit apart from being compensated for your time with a payment of £100 in Amazon gift vouchers (see http://www.amazon.co.uk for details). Travel expenses will also be reimbursed and refreshments will be provided. In addition, you will be offered a picture of your brain after your scan has been checked by our radiologist (approximately 2 months later) and we will send you a short report about some of the data we collected from you upon request.

What do I do if I want to withdraw from the study?
You are free to withdraw from the study at any time. You are not required to give us any reasons for withdrawal from the study but please inform us as soon as possible if you wish to do so.

Will my participation be kept confidential?
If you agree to take part in this study you will be identified in our computers by a number instead of your name. All records obtained while you are in this study will remain strictly confidential at all times. A copy of this ‘Information Sheet’ and of the signed ‘Consent Form’ will be given to you to keep. A copy of your consent may be made available to others working on the study at the Institute of Psychiatry and University of Southampton, and the Independent Ethics Committee members. More information on confidentiality is given in Part 2 of this information sheet.

If you have any questions about matters related to the study please contact Dr. Mitul Mehta on 020 3228 3058/3053.

PART 2
What will happen if I don’t want to carry on with the study?
If you withdraw from the study we will retain and continue to use any data collected before such withdrawal of consent unless you request that you do not want us to use any data collected from you.
If you require any support or counselling relating to your participation in this study our research team will be able to arrange this for you.

Will my information be kept confidential?
All information obtained during the study, as well as related health records, will remain strictly confidential at all times. However, these may need to be made available to others working on the Institute of Psychiatry or University of Southampton’s behalf or Ethics Committee members. For clinically significant findings highlighted in the study, we will notify your GP. Additionally, you will be informed about any unexpected findings. A face-to-face meeting can then be arranged and appropriate support provided.
By signing the consent form you agree to this access for the current study and any further research that may be done. However, the Institute of Psychiatry and University of Southampton will take steps to protect your personal information and will not include your name on any sponsor forms, reports, publications, or in any future disclosures. If you withdraw from the study, we will no longer collect your personal information, but we may need to continue to use information already collected. The study information collected may be sent to other locations outside of the UK, but you will not be referred to by name or identified in any report or publication nor could the information be traced back to you. This will be for healthcare and/or medical research purposes only. Your data will only be shared with countries where data protection laws are comparable to those in the UK. The Institute of Psychiatry and the University of Southampton maintain high standards of confidentiality and protection.

Under the data protection laws the University of Southampton is the controller of your personal data. Your anonymised data may be transferred within the University of Southampton, to others working on their behalf in the UK and the European Union (EU). The University of Southampton will take steps to ensure your personal data is protected and by agreeing to take part in this study you give your permission for these transfers.

You may withdraw your permission at any time by providing written notice to the study team. The study staff would then no longer use or share your personal information in connection with this study; unless it is essential to ensure that the study is scientifically reliable. However, we would still use study data that was collected before you withdrew your permission. In addition, you would no longer be able to participate in the study.

If you agree to take part in the study we may use the data collected in the following ways:
1. Your study data, either alone or combined with data from other studies, may be shared with collaborators from other countries in order to address scientific questions. Any data shared will be anonymized and will remain under the control of the sponsor.
2. Study data that does not identify you may be published in science journals or shared with others as part of scientific discussions.

You have the right to see and copy your personal information related to the research study for as long as the research institution holds this information. However, to ensure the scientific integrity of the study, you will not be able to review some of your personal information related to the study, until after the study has been completed. If any issues such as child abuse, neglect or other criminality come to light during the interviews, we are obliged to report this to the relevant authorities.

YOUR RIGHTS UNDER ANY APPLICABLE DATA PROTECTION LAWS ARE NOT AFFECTED

**What will happen to the results of the research study?**

The results of this research will be published as scientific reports and maybe presented at meetings within the Institute of Psychiatry or the University of Southampton or at international scientific meetings. You will not be identified in any report or publication that results from this study.

**Who is organising and funding the research?**

The research is being organised as a collaborative study between the Institute of Psychiatry, King’s College London and the University of Southampton, who is sponsoring the study. The study is funded by the Medical Research Council, UK. The researchers involved in conducting this study do not receive any financial incentives for including you in this study and do not benefit financially from this study.
Who has reviewed the study?

This research study has been looked at by two independent groups of people. The first is the Medical Research Council (UK) who determined the scientific merits of this study. The second is a Research Ethics Committee, who reviewed the study and documentation to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Camberwell - St. Giles Research Ethics Committee.

If you have any questions about matters related to the study please contact Dr. Mitul Mehta on 020 3228 3053/3058, Professor Edmund Sonuga-Barke on 02380 594580, or the King’s College London Research and Development Office on 0207 848 0251 or University of Southampton Research Governance Office on 02380595058.
Appendix D: Participant Consent Form.
Appendix

STUDY TITLE
English and Romanian Adoptees Brain Imaging Study

Principal Contact for general queries:
Dr Mitul Mehta
Centre for Neuroimaging Sciences, Institute of Psychiatry, London SE5 8AF
Telephone: 020 3228 3053

AGREEMENT TO PARTICIPATE

<table>
<thead>
<tr>
<th>Your Consent</th>
<th>Please tick</th>
</tr>
</thead>
<tbody>
<tr>
<td>01. I confirm I have read and understand the information sheet dated 04.08.2016 version 6 for the above study and have had the opportunity to ask questions.</td>
<td>☐</td>
</tr>
<tr>
<td>02. I understand that my participation is voluntary and that I am free to withdraw without giving any reason and at any time (including when inside the scanner), without my medical care or legal rights being affected.</td>
<td>☐</td>
</tr>
<tr>
<td>03. I understand that members of the research team at the Centre for Neuroimaging Sciences and the sponsor, the University of Southampton, will have access to the data from this study.</td>
<td>☐</td>
</tr>
<tr>
<td>04. I consent to the collection, processing, reporting and transfer of my anonymised data for healthcare and/or medical research purposes. The University of Southampton will confirm that any data transferred will be to countries with comparable data protection laws to the UK.</td>
<td>☐</td>
</tr>
<tr>
<td>05. I understand that any serious criminal activity that I discuss will be reported to the relevant authorities.</td>
<td>☐</td>
</tr>
<tr>
<td>06. I agree not to restrict the use of any data or results, which arise from this study.</td>
<td>☐</td>
</tr>
<tr>
<td>07. I agree to take part in the above study.</td>
<td>☐</td>
</tr>
<tr>
<td>08. I consent to the research team being informed of the results from any radiological review of my MRI scan data.</td>
<td>☐</td>
</tr>
<tr>
<td>09. I consent that any clinically significant findings will be reported to my GP.</td>
<td>☐</td>
</tr>
<tr>
<td>10. I consent to provide a sample of my DNA using a simple mouth swab.</td>
<td>☐</td>
</tr>
</tbody>
</table>

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Consent form 04.08.2016 version 5
Appendix E: Amygdala manual segmentation protocol.
Amygdala

Manual Tracing Methods

In use at:
NeuroImage Analysis Lab
University of North Carolina
Autism & Fragile X Research

Original Protocol developed by:
Center for Neuroscience and the M.I.N.D. Institute
University of California Davis

AMYGDALA TRACING: Introduction & Images:

- The images used in the following methods are T1 gray level images, aligned along the long axis of the hippocampus. Prior to applying these tracing methods, the original T1 images are aligned along the hippocampal axis and resliced to isotropic voxels (1.01562 mm^3) using the BRAINS2 software package (University of Iowa).
- At UNC, tracing is done with the IRIS/SNAP program. For coronal images from BRAINS2, the IRIS/SNAP file orientation is RIF. In IRIS/SNAP, voxels covered by 50% or more of the polygon you draw are included in the volume measurement.
- Note that in IRIS/SNAP you will trace the right amygdala on the left side of the screen and the left amygdala on the right side of the screen.

Overview of Steps:

1. Trace the amygdala in the coronal plane.
2. Check tracing in the axial plane and exclude the putamen.
3. Check tracing in the sagittal plane and define the rostral extent of the amygdala.
Appendix

Most Recent Update: November, 2003

Coronal tracing method:

- Find the most caudal section of the amygdala as it appears dorsal to the inferior horn of the lateral ventricle and hippocampus, and lateral to the optic tract. Begin by tracing the amygdala from the dorso-lateral extent of the optic tract.
- In caudal sections, the putamen forms the lateral border of the amygdala. If this border is seen, extend a line from the dorsolateral extent of the optic tract directly lateral (horizontal) to the amygdala/putamen border. If this border is difficult to see, extend the horizontal line laterally to the white matter. You can further define and exclude the putamen in the horizontal / transverse view (described later).
- Continue tracing the amygdala by following either the putamen/amygdala border, along the white matter, or (if all else fails) a line directly ventral (vertical) until the lateral ventricle is reached.
- The ventral border of the amygdala is initially formed by the lateral ventricle, then more rostrally by the hippocampus. The ventro-medial portion of the amygdala extends just ventral to the optic tract. More ventral, this border is formed by the amygdala-hippocampal transition area (which is included as part of the hippocampus). If this border is ambiguous, a division may be defined by a line perpendicular to the optic tract. More dorsal, the border is formed by the medial surface of the brain. Therefore, continue tracing the ventral surface of the amygdala along the ventricle, then amygdala-hippocampal transition area, medial surface of the brain, to the starting point at the dorso-lateral extent of the optic tract.
- Continue tracing the amygdala in more rostral sections as described above. When the medial surface of the brain extends further lateral than the optic tract, use the dorso-lateral extent of the medial surface as the dorsal border of the amygdala. Draw a straight horizontal line laterally from this point to the white matter. At this point, the putamen is no longer present and the lateral border is formed by white matter. Follow the curve of the white matter to the lateral ventricle.
- Further rostral, the hippocampus forms the ventral border of the amygdala, divided by a thin section of white matter (alveus). At this level, the amygdala-hippocampal transition area will no longer be present. Instead, the ventral border of the amygdala will be fairly horizontal (from the lateral border to the medial surface of the brain at the semiannular sulcus).
- If the border between the amygdala and hippocampus is difficult to find, look for the dorsomedial point of the lateral ventricle—if the ventricle is curving in medially, it will point to the alveus.
Appendix

As the hippocampus recedes medially in more rostral sections, the entorhinal cortex begins to form the dorso-medial border of the amygdala. The most dorsomedial point of the amygdala is at the semiannular sulcus on the medial surface of the brain.

Look for white matter to separate amygdala from entorhinal cortex medially. If this is difficult to see, then find the most medial point of the white matter (ventral to the amygdala) and draw a straight-line dorso-medially to the semiannular sulcus.

As the hippocampus and lateral ventricle disappear along the ventral border of the amygdala, white matter primarily forms the dorsal, lateral, and ventral borders. The medial border is formed by the entorhinal cortex and the dorsomedial border of the amygdala will be formed by the medial surface of the brain. Be careful to exclude vessels, which appear bright white in the image.

As you continue to trace the amygdala in rostral sections, the medial surface of the brain will extend further lateral (joining the lateral sulcus) to separate the temporal lobe from the rest of the brain. At this point, the dorsal border of the amygdala is defined by the surface of the brain.

Continue tracing the amygdala until it is indistinguishable. You can be generous here since the rostral border of the amygdala will be trimmed in the sagittal view.

Horizontal (transverse) view trimming:

- Begin at the most dorsal section of the amygdala and progress ventrally. The putamen may be found as an elongated tail extended caudally from the caudal portion of the amygdala. It may also appear a slightly darker gray. To exclude the putamen, follow the white matter along the lateral border of the amygdala as it extends caudally. Continue to draw a straight (medial-caudal diagonal) line, through the putamen, to the white matter on the medial side of the putamen just lateral to the thalamus. Further ventral, this line may terminate at the medial surface of the brain.

- Before continuing, review the rest of the tracing through the transverse sections and delete any scattered points. Do not bother smoothing out lines, as they will then appear jagged in the coronal view.

Sagittal view trimming:

- Begin by reviewing the amygdala on the most medial section you traced. To determine the rostral extent of the amygdala, follow the natural curvature of the ventral surface along the white matter as it extends rostrally. If possible, continue to follow the white matter to the
surface of the brain. If not, then follow the natural curvature from the most rostral tip of the white matter to the medial surface.

- Also, check the amygdala-hippocampal border in the sagittal view. The division between the amygdala and hippocampus appears as a diagonal line (approximately 45° dorso-caudal to ventro-rostral) from the dorso-caudal tip of the amygdala to the ventro-rostral tip of the hippocampus (often marked by a small portion of the lateral ventricle). This may be checked again after tracing the hippocampus.

- Review the rest of the trace through the sagittal sections and delete any scattered points. Do not bother smoothing out lines, as they will then appear jagged in the coronal view.

**Final check:**
Review amygdala tracing in the coronal view before calculating volume measurements.

**Summary Information**

**Tool used:** IRIS/SNAP manual tracing tool (UNC).

**Approximate time** to complete one case is 1.5 to 2.0 hours
This time includes tracing and thorough review of segmentation.

**UNC NeuroImage Analysis Lab reliability statistics for pediatric amygdala segmentations**

- Intra-rater for primary rater (average right and left) = 0.90
- Average inter-rater ICC (two raters, average right and left) = 0.78

Average inter-site ICC (two raters, average right and left) adult images = 0.92

<table>
<thead>
<tr>
<th></th>
<th>Right Amygdala</th>
<th>Left Amygdala</th>
<th>Average Right/Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-rater (rater A)</td>
<td>0.91</td>
<td>0.89</td>
<td>0.90</td>
</tr>
<tr>
<td>Intra-rater (rater B)</td>
<td>0.85</td>
<td>0.53</td>
<td>0.69</td>
</tr>
<tr>
<td>Average intra-rater</td>
<td>0.88</td>
<td>0.71</td>
<td>0.80</td>
</tr>
<tr>
<td>Inter-rater (ICC) for A &amp; B</td>
<td>0.86</td>
<td>0.69</td>
<td>0.78</td>
</tr>
<tr>
<td>Inter-site (with UC Davis)</td>
<td>0.94</td>
<td>0.89</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**References:**


UC Davis, Center for Neuroscience Amygdala Tracing Protocol.
Completed pediatric amygdala traces using IRIS/SNAP (right amygdala in green; left in blue).
Appendix F: Hippocampus manual segmentation protocol.
EADC-ADNI HARMONIZED PROTOCOL FOR MANUAL HIPPOCAMPAL SEGMENTATION:
USER MANUAL
Appendix II

1. Introduction

This document provides instructions to perform manual segmentation of the hippocampus on magnetic resonance images (MRI), as defined by the EADC-ADNI Harmonized Protocol for Hippocampal Segmentation (HarP) and described elsewhere [1]. Anatomical boundaries are described in detail with reference to pertinent publications relating to the hippocampus [2,3] and the human brain [4]. Reference information relative to the entorhinal/perirhinal cortex was taken from [5]. Additional material is available at www.hippocampal-protocol.net. Individual morphological variability may correspond to differences in the described landmarks. In this manual, some possible anatomical variants were considered, however the tracer should always check the anatomy of the segmented structures using an atlas of the human brain to ascertain the correct anatomical identification.

We suggest to reference the HarP by both the definition [1] and validation papers [6] whenever possible.

1.3 Abbreviations

MRI          Magnetic Resonance Imaging/Image
GM           Gray Matter
WM           White Matter
CSF          Cerebro-Spinal Fluid
HarP         Harmonized Protocol
SU           Segmentation Unit
AC           Anterior Commissure
PC           Posterior Commissure
EADC         European Alzheimer’s Disease Consortium
ADNI         Alzheimer’s Disease Neuroimaging Initiative
3D           Three-dimensional
2. Segmentation procedures

2.1 Image Orientation
The orientation of MRIs is determined on the sagittal view, by the line connecting the anterior and posterior commissures of the brain (AC-PC line) [7]. The coronal slices used for segmentation are resliced orthogonal to this plane.

2.2 Direction of segmentation
The segmentation is described and should be performed from rostral to caudal, on coronal slices.

2.3 3D navigation
The HarP requires the use of visualization tools allowing 3D navigation. For many landmarks, the morphological details visible in the coronal slice are not sufficient to determine whether the tissue belongs to the hippocampus. To perform accurate segmentation, the axial and sagittal planes must be checked frequently, and according to the advice reported in this HarP user manual.

3 Segmentation landmarks
The partition of the hippocampus into head, body and tail was not matter of decision of the Delphi panel. Since this kind of partition is useful to help describe the anatomical landmarks of this protocol, we use it as commonly found in current literature on the hippocampus. Here, we consider as hippocampal head the most caudal portion of the hippocampus, as long as it appears as a folded structure in the sagittal view, or as a double-layer structure in the coronal view. The level of the body includes, in rostro-caudal direction, the first slice where the hippocampus appears as a single, unfolded structure on the sagittal view and as a single- rather than double-layered structure in the coronal view. The level of the tail includes the last portion of the hippocampus, starting approximately at the level where the colliculi can first be visualized in the coronal view.

3.1 Most rostral slice
The most rostral slice where the hippocampus is segmented is the first slice where some hippocampal tissue is visible below the amygdala in the coronal view, and after checking in the 3D (axial and sagittal planes). The very first tissue that can be detected with the most accurate 3D navigation is the alveus, a thin WM layer that covers the GM of the hippocampal head. The alveus may not be clearly visible in the coronal plane in some slices – depending on signal to noise and...
contrast properties of a particular MRI scan. Therefore, the segmentation of hippocampal tissue on
the most rostral slices needs 3D navigation to discriminate the continuous hyperintense line of the
alveus on the dorsal border of the hippocampus in the sagittal view (Figure 1). An inlet of CSF can
be discriminated between the hippocampal head and the amygdala in the sagittal and axial planes
(Figure 1, arrows).
The GM located below the alveus, and confirmed to belong to the hippocampus from the sagittal
views and axial views, is included in the segmentation. All of the GM located above the alveus is
excluded as belonging to the amygdala. The alveus is included in the segmentation. Sometimes the
first slice only shows the WM of the alveus; if this can be distinguished with confidence, using both
the coronal and sagittal planes, this WM should be included in the segmentation as the very first
slice.
Please note that, especially in lower field strength images, intensity inhomogeneities in this region
may be mistaken as the alveus, but actually belong to amygdalar tissue. Gray scale intensity
differences alone do not distinguish which voxels belong to the hippocampus or the amygdala.
For this reason, checking in 3D is always necessary. In the coronal view, the digitations of the
hippocampal head at this level make an undulating dorsal boundary. However, the sagittal and axial
views usually show smoother boundaries, and clearer contours of the alveus and the CSF, that aids
the anatomical identification of tissue.
Figure 1. Identification of the most rostral hippocampal tissue. Coronal view: first slice where hippocampal tissue can be detected and segmented. This may consist of the alveus WM only. Sagittal and Axial views: Segmentation of the most rostral coronal slice must be ascertained in 3D, where the continuity of the WM line of the alveus can be seen in the sagittal view, and where the CSF inlet separating the hippocampal head from the amygdala can be clearly seen in the axial view (arrows; in the right panel the target is segmented in yellow to highlight the exact structure pointed by the arrow).
3.2 Most caudal slice

The most caudal slice where the hippocampus is segmented is the last one, in the rostro-caudal direction, where a small ovoid GM mass is visible inferomedially to the trigone of the lateral ventricle (Figure 2).

**Figure 2.** Last coronal slice where the hippocampus can be detected and segmented. The vertical line in the sagittal view corresponds to the level shown in the coronal view. Based on the criteria of inclusion of the hippocampal WM (alveus and fimbria, see section 3.4.4), the WM adjacent to the hippocampal GM included in the segmentation must also be included.

3.3 Ventral boundary

The ventral boundary of the hippocampus is defined by the WM of the parahippocampal gyrus throughout the whole structure.

3.4 Dorsal boundary

Depending on the level (head, body, tail), on individual morphology, and on the image quality, the dorsal boundary of the hippocampus is defined by the most dorsal boundary of the hippocampal GM bounded by CSF, or by the most dorsal boundary of the alveus and fimbria (to be included in segmentation, see section 3.4.4), whenever present and visible.

3.4.1 Dorsal boundary at the level of the head
In the most rostral slices, the neighboring tissue is the GM of the amygdala. Determining the dorsal boundary is aided by using 3D navigation. In particular, the sagittal view enables identification of the folded shape of the hippocampal head (Figure 3, sagittal view). Both the sagittal and the axial views show the CSF separating the hippocampal head from the amygdala, and provide a better visualization of the alveus.

**Figure 3.** Separation of the hippocampal head (yellow segmentation on the left) from the amygdala (red dotted segmentations) at the level of the vertical digitation of the hippocampus. The yellow asterisks denote the vertical digitation, that must be included in the segmentation. The sagittal view shows the clearer separation of the hippocampal head from the amygdala. The separation between the two structures is very clear due to the visible folded shape of the hippocampal head and the CSF inlet separating it from the amygdalar tissue.

The boundary between the hippocampal head and the amygdala is an issue not only for the most rostral hippocampal tissue, but for many slices along the hippocampal head. When the hippocampal head is not separated from the amygdala (Figure 4a and 4b), the dorsal boundary consists of the WM of the alveus/limbria (to be included in the segmentation).
When the CSF inlet from the lateral boundary with the lateral ventricle (see Figure 4: "4 – Temporal horn of the lateral ventricle") extends medially, separating the hippocampus from the amygdala, the dorsal border is partly defined by this interface with the CSF of the temporal horn of the lateral ventricle. At this level, the amygdala extends into a vertical-oblique band of tissue containing its most caudal accessory basal and cortical nuclei (Figure 4c. See also [2] at page 135: Figure 7.5 b). This oblique band belonging to the amygdala must be excluded from the segmentation. At this level, therefore, the dorso-medial border of the segmentation consists of the border with these last nuclei of the amygdala. In this region, it is usually not possible to detect voxel intensity differences helping boundary discrimination, therefore this boundary must be inferred through very careful examination of the involved structures using the 3D visualization and relevant atlases (Figure 4; [2] at page 131: Figure 7.4 b, 135: Figure 7.5 b, 139: Figure 7.6 b). It must be underlined that this is a very frequent source of mistakes, deserving great attention for correct anatomical segmentation.

In more caudal slices of the head, when the amygdala appears as progressively more buried in the above temporal stem above, (Figure 4d, see also red arrow in Figure 3), the dorso-medial hippocampal tissue consists of the end of the vertical digitation of the hippocampus, that must be fully included in the segmentation (Figures 3 and 4d). Please note also that the apical tissue of these last slices of the hippocampal head (where the amygdala is buried in the temporal stem, as 3 in Figure 4d) is a frequent source of mistakes: in these slices, all of the apical tissue of the vertical digitation must be included in the segmentation (see “correct segmentation” in Figure 3), as confirmed by the sagittal view during 3D navigation. At this level, the neighbouring region to be excluded from segmentation is only the CSF, and the small boundary with the amygdala, running continuously with the profile of the temporal stem.
Figure 4. Neighbouring structures at the level of the hippocampal head.

1 - Hippocampus
2 - Alveus/Fimbria
3 - Amygdala
4 - Temporal Horn of the Lateral Ventricle
5 - Parahippocampal Cortex
6 - Vertical Digitation
7 - Posterior Cerebral Artery
3.4.2 Dorsal boundary at the level of the body and tail
At the level of the body and tail, the dorsal border of the hippocampus is the interface of hippocampal tissue (GM, or alveus/fimbria in the dorsolateral aspect) with the neighbouring CSF.

3.4.3 Exclusion of the choroid plexus
At the level of the hippocampal body and tail, the choroid plexus of the ventricle extends along the dorsal aspect of the hippocampus. The choroid plexus consists of capillaries, separated from the ventricles by choroid epithelial cells, which filter liquids from the blood to generate CSF. It is found in temporal horn of the lateral ventricles, and must be excluded from the hippocampal segmentation. Distinguishing the hippocampal tissue from the choroid plexus may be difficult since the two structures are characterized by a similar gray intensity on MRI. However, the choroid plexus appears less dense than the hippocampus, looking similar to an inflorescence. The alveus/fimbria can usually be visualized at this level, allowing the segmentation of the dorsal border, and the exclusion of the choroid plexus located above.

When the alveus/fimbria is not visible, and the choroid plexus is dense and therefore visually similar to hippocampal GM or WM, it can be recognized through 3D navigation as being detached from the hippocampus along its length (red asterisk in Figure 5).
3.4.4 Inclusion of the alveus/fimbria

The alveus-fimbria-fornix pathway is one of the major conduits for subcortical afferent and efferent connections. The fibers of the alveus/fimbria are continuous with the fornix; these structures take different names based on their location.

The ventricular surface of the hippocampus is covered by a thin layer of myelinated fibers called “alveus”. More caudally, the fibers of the alveus extend obliquely, from lateral to medial, on the hippocampal surface, collecting in the thicker bundle called “fimbria”. The fornix is the continuation of this bundle, detaching from the hippocampus to reach the target subcortical structures. At the post-commissural level, as these WM fibers depart from the hippocampus, they are named “crus” and “column of fornix” ([3], page 47; [2], Figures 7.15 d-e, 7.16 d-e, 7.17 d-e, 7.18 c).