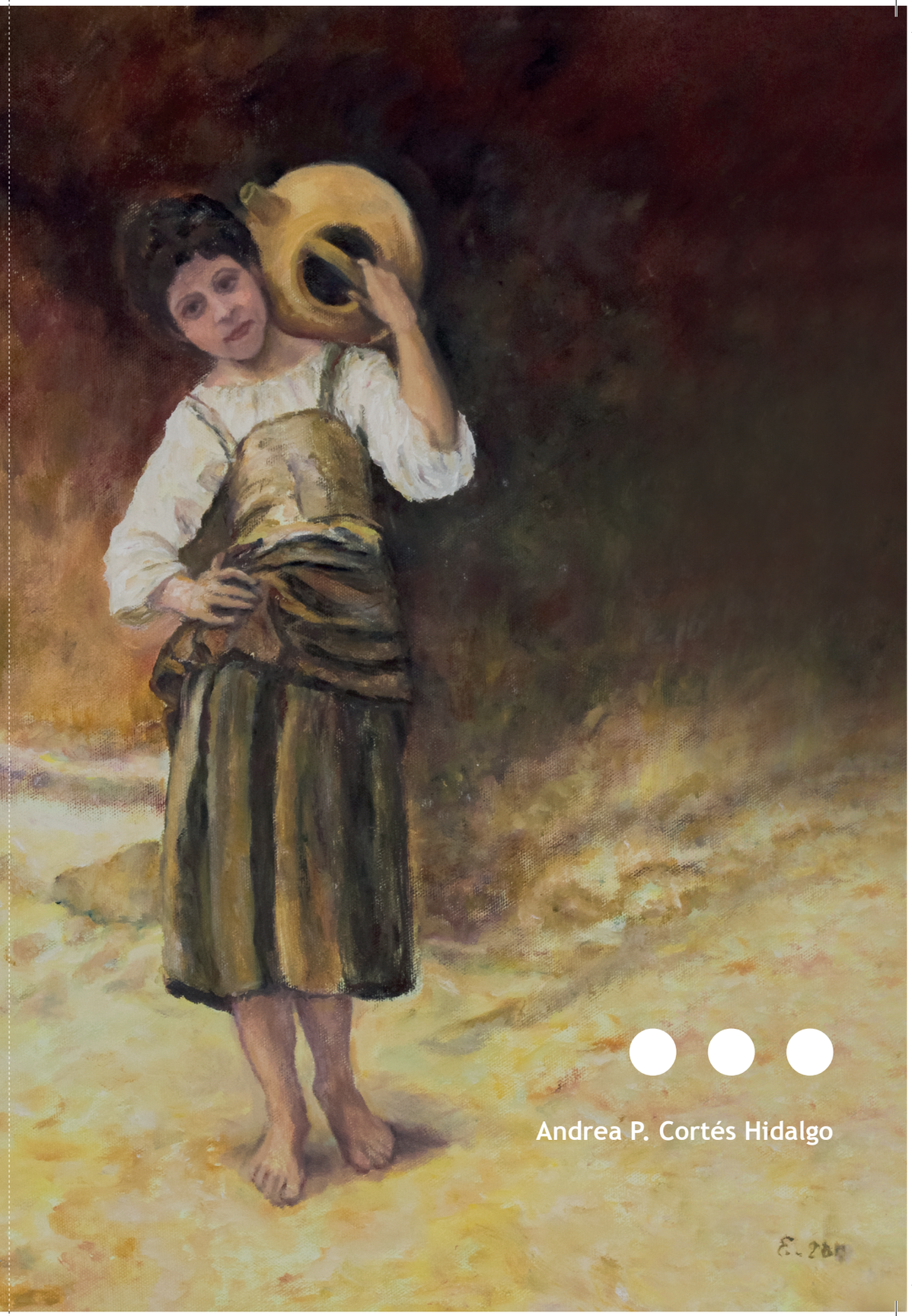
Early-Life Adversities and Neurocognitive Outcomes *An Epidemiological Study*

Andrea Patricia Cortés Hidalgo



Andrea P. Cortés Hidalgo

Early-Life Adversities and Neurocognitive Outcomes

An Epidemiological Study

Andrea Patricia Cortés Hidalgo

The work presented in this thesis was conducted at the Department of Child and Adolescent Psychiatry/Psychology and the Generation R Study Group, Erasmus Medical Center, Rotterdam, the Netherlands. The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, the Rotterdam Homecare Foundation, and the Stichting Trombosedienst and Artsenlaboratorium Rijnmond (STAR-MDC), Rotterdam, the Netherlands.

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Early-Life Adversities and Neurocognitive Outcomes
An Epidemiological Study

Tegenslagen in de Kindertijd en Neurocognitieve Ontwikkeling
Een epidemiologische studie

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
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and in accordance with the decision of the Doctorate Board.
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by

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Co-promotor:	Dr. T.J.H. White

Paranymphs	Ellis Voerman Runyu Zou

FROM INTRODUCTION TO COINCIDENCES
—PART I

Look at the line of time.

Of course, it is only an illusion. Time is a space, not a line.

But for our purposes, look at the line of time.

Watch it. Identify how each event on the line is both a cause and effect. Try to locate its starting point.

You will not succeed, of course.

Every now has a before.

This is probably the main—though not the most obvious—problem you will encounter as coincidence makers.

Therefore, before studying theory and practice, formulas and statistics, before you start to make coincidences, let's start with the simplest exercise.

Look again at the line of time.

Find the correct spot, place a finger on it, and simply decide: "This is the starting point."

From: The Coincidence Makers, Yoav Blum
Cited with permission from the author

To my parents,
who taught me how to trust in God,
how to fly,
and how to work hard.

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1

Introduction

When was the last time you felt uncertain about your future, with no control over your work or too challenged by the circumstances?

Stress is a sensation that we all recognize and have experienced. The concept of stress is, however, broad and extremely difficult to define. In general, psychological stress occurs when a person perceives the environmental demands to be exceeding their own adaptive capacity (Cohen et al., 2007). However, rather than assessing the stress perception per se, most studies operationalize stress based on the occurrence of adverse events that are generally judged to be stress-provoking, such as physical abuse, war exposure, limited family resources or lack of cognitive stimulation (Smith & Pollak, 2020). This event-oriented approach is motivated by the goal of obtaining a more objective and clear measure of the stress exposure (Cohen et al., 2007).

Given the diversity of adverse events, multiple approaches have been proposed to organize adversity measures. In general, two types of approaches can be distinguished: the *lumping* and the *splitting* (Smith & Pollak, 2020). The first group supports the assessment of many different adverse events at the same time, with the assumption that the general exposure to stress is what matters, rather than the specific type of event. Within this framework, all events are considered to have relatively similar effects on the individual (Smith & Pollak, 2020). The *splitting* approach, in contrast, proposes that different types of events lead to different outcomes and categorizes adverse events into separate groups based on features presumed to be common. For example, the model of threat and deprivation distinguishes the exposure to direct threats (e.g. physical and sexual abuse, violence in the community) from the lack of expected inputs (e.g. neglect, institutional rearing, parental absence) (McLaughlin et al., 2019). Note, however, that the practical utility of the threat and deprivation categories is questioned by some scholars, because threat and deprivation very often co-occur (Pollak & Smith, 2021). Additionally, some studies examine single adverse events or experiences considered particularly relevant, such as natural disasters (Jones et al., 2019). These approaches are theoretically complementary. Whereas the *lumping* approach addresses the relevance of the association and offers a more naturalistic view of the occurrence of adversity (because adverse events rarely occur in isolation) (Smith & Pollak, 2020), the *splitting* approach aims to reveal specific mechanisms underlying the effect of adverse events. In the studies included in this thesis, we largely applied the *lumping* approach, but examined in detail some specific adversities in Chapter 3 and 5.

And have you ever wondered about whether stress can change your brain?

Very interesting work has shown that the brain can have local changes throughout life, adapting to environmental factors. For example, Maguire et al. (2000) found that the posterior region of the hippocampus was larger in taxi drivers compared to controls, and

the hippocampal volume positively correlated with the time spent as a taxi driver. Also, *changes* in the white matter and grey matter density were documented in adults who spent 6 weeks learning how to juggle (Scholz et al., 2009), suggesting that the brain can change in response to relatively recent events. Importantly, and specifically regarding stress, animal studies demonstrated that stressful events may have a causal relation with the structural remodeling of the hippocampus, amygdala and prefrontal cortex, as well as with specific neuronal alterations (Schiavone et al., 2013), therefore supporting the hypothesis that stress can in fact shape the brain.

Addressing this research question in humans becomes particularly relevant from a neurodevelopmental perspective. This is mainly because stress occurring while the brain develops is expected to have strong and long-lasting effects, and evidence identifying biological mechanisms through which early-life adversity may influence physical and mental health outcomes is critical to improve our understanding of the adversity effects and to develop public health interventions (Danese & Lewis, 2021; McLaughlin et al., 2019). Moreover, childhood adverse events are common, with a prevalence in the general population of up to 50%, depending on the events assessed (McLaughlin et al., 2019). Also, a relation between early-life adversity and subsequent psychological and cognitive outcomes is well documented (Hanson et al., 2017; Humphreys & Zeanah, 2015; Wesarg et al., 2020), supporting a link of adverse events experienced early in life with children's neurodevelopment.

Early-life adversity and brain morphology

Research has typically focused on severe cases of maltreatment (e.g. children identified by the Child Protective Services as physically abused) and neglect (e.g. institutionally-reared children). In general, most of these studies comprise small samples and cross-sectional designs (McLaughlin et al., 2019), but overall, findings support a relation between childhood adverse experiences and brain morphology (Riem et al., 2015). For example, Hanson et al. (2015) found smaller amygdala volumes in three different samples of children when compared to controls: institutionally-reared children, children from low SES (socioeconomic status) households, and children who were physically abused. Further, a randomized-controlled trial in institutionalized children showed white matter volume differences when comparing the children randomized to remain in the institution vs a group of never institutionalized children, but not when comparing the children randomized to foster care vs the never institutionalized children (Sheridan et al., 2012). Although limited by the small sample size and noticeable attrition (Nelson III et al., 2007), this study supports the link between early-life adversity and brain morphology and offers a particularly intriguing insight into the possibility of (partial) recovery after experiencing adversity. Interestingly, children who were randomized to foster care also showed better cognitive outcomes (Nelson III et al., 2007).

It is worth noting that although existing research supports an association between adversity exposure and brain morphology, the direction of the findings is conflicting for some of the brain structures assessed. Reduced total brain volumes, with differences in the gray and white matter volumes, have been relatively consistently reported in children exposed to adversity, but mixed results have been described for the volumes of the amygdala and hippocampus (see for a review: Bick and Nelson (2016)). Importantly, very few studies have examined the relation between childhood adversity and brain morphology in children from the general population; most work focused on severe adversities and high-risk groups. The studies in this thesis address this research gap with evidence based on a population-based sample.

Another important phenomenon that has received little attention is the occurrence of adverse events during pregnancy. Brain development starts very early in fetal life (White, 2019) and it is well known that a broad range of events/environmental factors experienced by the pregnant mother may have long-lasting consequences on the offspring, and could even contribute to the development of adult disease (Wadhwa et al., 2009). Despite the importance of this developmental period, very few studies have assessed the relation between events experienced by the mother during pregnancy and child brain development. One particularly interesting study assessed the exposure to a natural disaster. Jones et al. (2019) examined a group of 68 children whose mothers were exposed to an ice storm during pregnancy. This storm was so severe that resulted in electrical power failures during the coldest time of the year and was even referred to as the costliest natural disaster in Canadian history (Laplante et al., 2008). Researchers found that the degree of hardship experienced by the pregnant mothers was related to larger amygdala volumes at age 11 years and the amygdala volumes were also associated with more externalizing problems (Jones et al., 2019). To date, the relation between more common adverse events experienced by mothers during pregnancy and the child brain morphology is still under-studied.

Finally, in the study of early-life adverse events and child neurodevelopment, there are several points that need to be discussed. First, research on the effects of early adversity needs to consider the specific brain developmental period. Brain development begins early in fetal life and continues throughout childhood, including a series of delicate and intricate processes like the neuronal migration and the formation of synapses (White, 2019). Most brain structures increase in volume rapidly during infancy and by age 5, the brain size has reached about 90% of the adult size (Lenroot & Giedd, 2006). Given that brain development starts in embryonic life, it is important to examine exposure to adversity in pregnancy *and* in childhood to understand whether adversity exposure in specific developmental periods has stronger associations with the brain development than exposure in others.

Second, brain morphological differences resulting from adversity exposure could reflect a resilient neurobiological adaptation, that allows the individual to adjust to the adverse environment (Thijssen et al., 2017), or a pathological adaptation that leads to harmful consequences, such as the development of psychopathology (McLaughlin et al., 2020). Consequently, studies offering insights into neurobiological resilient adaption to adversity are needed. Until now, very few studies have assessed the neural convergence points of resilience from a neurodevelopmental perspective (Holz et al., 2020).

Aims of this thesis

The studies described in this dissertation had three aims: First, to investigate whether there is a relation between early-life adversities and child cognition and brain morphology in the general population. Second, to examine the association of protective factors with child brain morphology. Third, to address whether protective factors modified the relation between childhood adversities and the brain structure. Our hypotheses on these three aims are described in detail in each chapter.

Setting

Most studies in this thesis were performed using a population-based cohort, the Generation R Study (Kooijman et al., 2016). Designed to study the growth, development and health of children, the Generation R Study offers a unique opportunity to prospectively assess the association between early-life adversity and the child neurocognitive outcomes. In total, 9,778 mothers residing in Rotterdam with a delivery date between April 2002 and January 2006 were enrolled in the study (response at baseline 61%), and data was collected from children and parents through questionnaires, visits to the research center and visits to the participants' houses. This thesis makes use of the Generation R data collected from very early pregnancy onwards until the age of 10 years, when the brain magnetic resonance imaging scans were acquired. Although children exposed to extreme adverse events are included in this study, the majority of children who were exposed to adversity experienced common adverse events that are less severe.

Data from the Mannheim Study of Children at Risk (MARS) was included together with data from the Generation R Study, in **Chapter 8**. The MARS study was specifically designed to examine the long-term outcomes of early psychosocial and biological risk factors and included 362 infants born between February 1, 1986 and February 28, 1988 in the Rhine-Neckar region of Germany (Laucht et al., 2000). In this cohort, data was prospectively collected on children and their parents, and brain images were obtained at age 25 years (Monninger et al., 2019).

Outline

The research question of whether childhood adversities and early-life stress are associated with child neurocognitive outcomes is addressed in **Section A**, using different methodological and theoretical approaches to stress. In **Chapter 2** we present a study on prenatal maternal stress, modelled with a latent construct. In **Chapter 3** we focus on the exposure to harsh parenting, reported by mothers and by fathers. In **Chapter 4** we explore both prenatal and childhood cumulative adversities in relation to child brain outcomes. In **Chapter 5** we focus on two adversities: threatened and actual violence exposure in childhood. In **Chapter 6** we address the exposure to family poverty from fetal life onwards.

In **Section B**, we evaluate the role of potential protective factors in relation to brain outcomes. **Chapter 7** presents the relation between infant-parent attachment and brain morphology, and **Chapter 8** describes the interplay between early-life adverse events, protective factors and subsequent measures of brain morphology. The moderation effect examined in the latter chapter helped us to explore the neurobiological underpinnings of resilience.

Finally, the implications of findings from studies described in this thesis, a discussion of methodological challenges, and considerations for future research are presented in the general discussion in **Chapter 9.**

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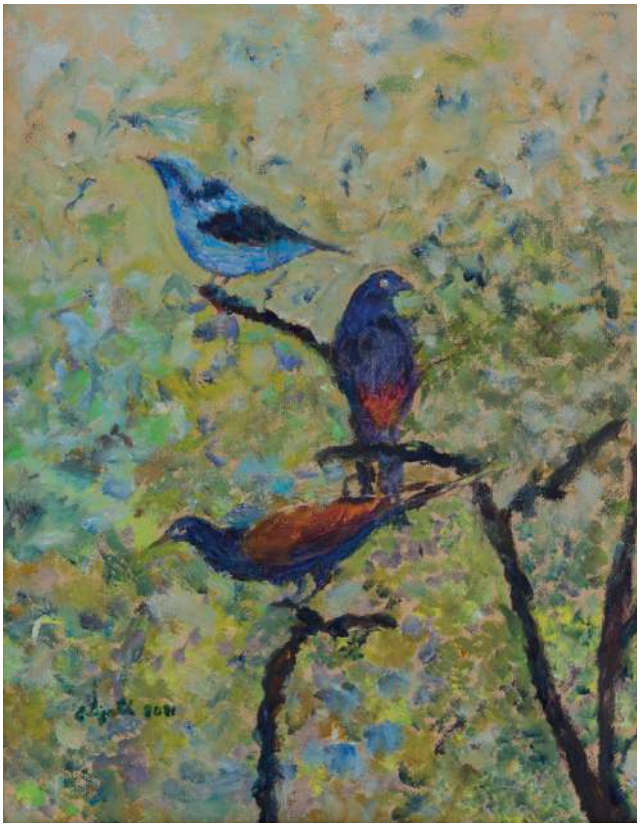

Section A

Adverse life events and child neurocognitive outcomes

2

Prenatal Maternal Stress and Child IQ

Cortes Hidalgo, A.P., Neumann, A., Bakermans-Kranenburg, M.J., Jaddoe, V.W., Rijlaarsdam, J., Verhulst, F.C., White, T., van IJzendoorn, M.H. and Tiemeier, H.

ABSTRACT

The evidence for negative influences of maternal stress during pregnancy on child cognition remains inconclusive. This study tested the association between maternal prenatal stress and child intelligence in 4,251 mother-child dyads from a multi-ethnic population-based cohort in the Netherlands. A latent factor of prenatal stress was constructed, and child IQ was tested at age 6 years. In Dutch and Caribbean participants, prenatal stress was not associated with child IQ after adjustment for maternal IQ and socioeconomic status. In other national origin groups no association was found; only in the Moroccan/Turkish group a small negative association between prenatal stress and child IQ was observed. These results suggest that prenatal stress does not predict child IQ, except in children from less acculturated minority groups.

INTRODUCTION

Fetal neurodevelopment represents a vulnerable period in which maternal exposure to stress is suggested to have a long-term impact on the development in offspring (Wadhwa et al., 2001). However, its impact on child cognitive outcomes is unclear. One of the reasons for inconsistent study results may be that the concept of maternal stress during pregnancy is imprecisely and differently defined; most previous research encompassed only one dimension as the measure of stress, such as psychopathology or stress reactions to *specific* events, and key confounders such as maternal IQ were often not taken into account. In this study, we assessed maternal prenatal stress as a latent construct based on several manifestations of stress in different life domains. Our population-based prospective cohort study gave us the opportunity to examine the association between stress during pregnancy and child intelligence in the offspring of mothers with various national origin backgrounds and lifestyle characteristics.

Maternal stress during pregnancy and offspring cognition

Barker et al. (1986) hypothesized that maternal undernutrition during pregnancy causes fetal changes with long-term consequences in the offspring. While this theory was initially developed in relation to maternal undernutrition, it was later broadened to include other in-utero exposures. This comprehensive model, termed as the "Developmental Origins of Health and Disease" (DOHaD), proposes that the environment can have a long-lasting influence during the phase of developmental plasticity, and in interaction with genetic factors determine health and risk of disease in later life (Gluckman & Hanson, 2006; Wadhwa et al., 2009). As part of the DOHaD model the effects of prenatal psychological stress on offspring developmental outcomes have also been evaluated, with the purpose of understanding the relation between maternal stress and fetal development as well as later psychobiological outcomes (Wadhwa et al., 2009).

Studies that assessed maternal stress during pregnancy in relation to offspring outcomes have used different definitions of stress. Stress is thought to occur when individuals perceive the environmental demands as exceeding their capacity of adaptation (Cohen et al., 1995). This broad definition of stress has led to studies of a wide variety of stressors in pregnant women, such as interpersonal problems, financial difficulties, physical complaints, depression, or worries about their pregnancy (O'Donnell et al., 2009). Also, while many scholars consider depression and stress to be different concepts, studies on the association of prenatal stress and child outcomes often include depression in the stress definition. For example, a systematic review of the association between maternal prenatal stress and young children's cognitive development operationalized maternal psychological distress as the occurrence of depression, anxiety, perceived stress or stressful experiences during pregnancy (Kingston et al., 2015). Very similar

inclusion criteria were used by Kinsella and Monk (2009) in their narrative review of maternal stress studies, which summarized the associations of maternal depression, stress, and anxiety with neurobehavioral outcomes. Pregnancy-specific anxiety has also been included as part of the maternal prenatal stress concept in studies on the association with child mental development (DiPietro et al., 2006). The variation in the definition and measures of maternal prenatal stress reflects that stress during pregnancy can manifest differently in different domains of life. In the following section, we briefly summarize studies focusing on specific stress measures. Studies are organized according to their measure of stress to facilitate a review of the literature.

Maternal anxiety during pregnancy

Studies of prenatal maternal anxiety and child cognition show inconsistent results. Brouwers, Van Baar & Pop (2001) observed an association between prenatal anxiety, as measured with the State-Trait Inventory, and less optimal offspring mental development assessed with the Bayley scales of Infant Development in a group of 105 2-year old Dutch children. Likewise, higher maternal prenatal anxiety was associated with lower IQ scores in a sample of 57 adolescents aged 14 to 15-year old (Van den Bergh et al., 2005) and with lower academic performance in 5,801 16-year old adolescents in the ALSPAC cohort (Pearson et al., 2016). In contrast, Grant et al. found no difference between infants of mothers with prenatal anxiety and controls in their scores for the mental development index of the Bayley scales in a sample of 77 7-month old children (Grant et al., 2010). Similarly, Koutra et al. found that maternal anxiety during pregnancy did not predict less optimal cognitive development in offspring using a sample of 223 18-month old children from a population-based cohort in Greece (Koutra et al., 2013). DiPietro et al. (2010) reported that higher levels of maternal prenatal stress were related to accelerated fetal and infant neurological maturation in a sample of 112 healthy pregnancies. Also higher levels of maternal prenatal anxiety were associated with *better* offspring mental development measured with the Bayley Scales of Infant Development in 82 2-year old children that belonged to a well-nourished, financially stable population (DiPietro et al., 2006).

Maternal depression during pregnancy

Studies of prenatal maternal depression and child cognitive outcomes also showed discrepant results. Self-reported depressive symptoms were assessed in a sample of 6,979 pregnant mothers from the ALSPAC cohort and children of mothers with higher levels of depressive symptoms during pregnancy had slightly worse cognitive functioning as measured by the WISC (Evans et al., 2012). In contrast, Tse et al. observed that children who were exposed to maternal depression during pregnancy did not perform

differently, compared to non-exposed children, on the Peabody Picture Vocabulary Test (PPVT) at 3 years of age in a study of 1,030 mother-child pairs (Tse et al., 2010).

Other measures of prenatal stress

A few studies assessed maternal perceived stress during pregnancy, i.e., the degree to which life events were considered stressful. Perceived maternal prenatal stress was related to lower offspring intelligence as measured with the Stanford Binet Scale in 550 3-year old children (Slykerman et al., 2005). Other studies related pregnancy-specific anxiety to child cognitive outcomes. Huizink et al. studied pregnancy-specific anxiety in a sample of 170 mothers, and found that higher self-reported pregnancy-specific anxiety predicted lower mental developmental scores as measured with the Bayley Scales of Infant Development in 8-month old children (Huizink et al., 2003). Similar results were observed by Davis et al., who studied the presence of pregnancy-specific anxiety in a sample of 125 pregnant mothers and found an association with lower mental scores on the Bayley scales in 1-year old children (Davis & Sandman, 2010). Laplante et al. studied a group of 89 mothers, who were exposed during pregnancy to an ice storm in the Canadian province of Québec. They observed that maternal prenatal stress, retrospectively reported, was associated with lower children's IQ scores at age 5 years (Laplante et al., 2008).

A broad definition of stress

The previous studies aimed to investigate how specific aspects of maternal stress are related to child cognitive development. However, stressful events rarely happen in isolation but rather tend to co-occur, increasing the risk for a deleterious offspring effect (Appleyard et al., 2005). Moreover, psychological or perceived stress cannot be directly observed (Milfont & Fischer, 2010) and can only be assessed by self- reported indicators that represent related aspects of stress (for example, daily hassles, severe life events) (O'Donnell et al., 2009). The substantial conceptual and phenotypic overlap of these stress measures are arguments supporting a broad concept of perceived stress. Further, no specific mechanistic pathways towards offspring cognition have been established for any of the different perceived stress measures. Therefore, to examine the broad concept of perceived prenatal maternal stress, we constructed a latent variable. The latent variable model captures the structure underlying the covariance among the observed variables (the self-reported stress measures) (Bartholomew et al., 2011), while simultaneously reducing the dimensionality. This approach has an additional advantage. Members of different groups (e.g. females and males, old and young age groups, ethnic groups) are often compared with the assumption that pertinent variables represent similar constructs across groups. This assumption, known as measurement invariance, is often not tested (Milfont & Fischer, 2010). However, when there is lack of measurement

invariance (when the concept is not equivalent in all groups), the interpretation and comparison of the construct (prenatal maternal stress) across groups is not meaningful. Latent variable models can be specified without this assumption if the different groups are accounted for and give insight into the extent of variability of the construct between the groups.

We modelled a broad concept of stress to account for the high co-occurrence of stress factors. This broad stress definition, that encompasses different domains, allowed us to better examine the long-term effects of prenatal stress on child cognition than an individual stressor approach. We tested the measurement invariance of the stress concept in our multi-ethnic population-based sample. Due to lack of invariance across national origin groups, we performed our analyses in separate national origin groups.

Methodological considerations

The studies on maternal stress and child cognition are not only characterized by a diversity of exposure measures but also vary in the degree to which they account for methodological challenges inherent to studies of prenatal exposure and child cognition. First, some researchers assessed maternal prenatal stress retrospectively (Laplante et al., 2008). Studies with retrospective stress assessment yielded larger effect sizes, which could reflect a problem of recall bias (Tarabulsy et al., 2014). Second, the existing literature of maternal prenatal stress and offspring cognitive outcomes includes a broad array of outcome measures in infants, children and adolescents, hampering a direct comparison of results. A meta-analysis of studies that assessed child cognitive development between 0 and 60 months of age (typically with the Bayley Scales of Infant Development) reported a small negative effect of prenatal stress (Tarabulsy et al., 2014). The literature in children between 5 and 12 years is less extensive and also shows less consistent results. Most studies examined the effect of specific stress measures and while some found a negative effect of prenatal stress (assessed as depressive symptoms, anxiety, or stress), others reported no association with child IQ. In contrast, research in adolescents shows a consistently negative effect of prenatal stress on child cognition. However, this evidence should be interpreted with caution as it is based on few, small studies and different specific cognitive measures, while IQ was rarely examined. Third, some studies lack adjustment for key confounders like parental intelligence. Cognitive ability is one of the most heritable traits (Polderman et al., 2015) and shared genetic effects could underlie any observed association between maternal stress and child cognition. Yet, parental intelligence is often not controlled for when examining the association between prenatal stress and child IQ. Fourth, parental education as a measure of genetic transmission and environment quality (i.e. socioeconomic status and intellectual stimulation) (Rowe et al., 1999) is strongly related to maternal stress and to child cognitive development. The lack of adjustment for parental education may make

it difficult to distinguish between the specific effects of prenatal stress and contextual influences on child cognitive outcomes. Most studies adjusted for parental education level; in these studies results are mixed. In contrast, studies that did not account for parental education show a consistent negative association between prenatal stress and child cognition. Fifth, stressed pregnant women tend to be stressed mothers. Postnatal stress influences caregiving and this could inappropriately augment the association between prenatal stress and child IQ. Lastly, the studies on the association of prenatal stress and child cognitive development do not usually take into account vulnerable groups, such as national origin minorities. Modern societies are characterized by large groups of national origin minorities, which are more likely to have financial difficulties and are vulnerable to experience stress related to acculturation due to stigma, prejudice and discrimination. This particular kind of stress has been described as 'minority stress' (Marshal et al., 2008; Meyer, 2003), and it is suggested that the social stressful environment experienced by these individuals generates a higher risk of mental health problems (Meyer, 2003). Also, although ethnicity and SES are often strongly intertwined, there are unique risks related to ethnic minority status that are not accounted for by SES, such as the social community networks, the degree of acculturation, and discrimination. (Dyal & Dyal, 1981; Williams, 1996).

The present study fills several gaps in the literature. We present evidence on the joint effect of different stress domains on the IQ of school-aged children. In our sample, stress was examined prospectively at different time points during pregnancy, child IQ was assessed with a non-verbal test when children were 5 to 7 years old, and we controlled our analyses for maternal education and IQ. Furthermore, we provide evidence on the role of national origin in the association between prenatal stress and offspring IQ.

Aim of the present study

The aim of the present study was to examine the association between global maternal prenatal stress and offspring IQ at age 6 years. We hypothesized that maternal stress during pregnancy is related to offspring intelligence, even after accounting for key confounders like maternal intelligence. We tested this hypothesis using structural equation modelling, assessing global maternal prenatal stress with a latent construct and child cognition with a non-verbal IQ test. Data were collected within the prospective multiethnic cohort of the Generation R Study.

METHODS

Setting and population

This research was conducted within the framework of the Generation R Study, a population-based cohort in Rotterdam, the Netherlands (Kooijman et al., 2016). This city has a larger proportion of ethnic minorities (44% of inhabitants are of foreign background) than the Netherlands (19%). The largest minority groups in Rotterdam are the Surinamese (9%) , Turkish (7%) and Moroccan (6%) (Statistics Netherlands, 2004). Mothers with a delivery date from April 2002 until January 2006 were enrolled to study determinants of early development and health. Among all eligible children, the response rate was 61%. The proportion of minority groups in our cohort was higher than nationally reflecting the urban setting of the study, but differed only marginally from that in Rotterdam. The largest minority groups were the Surinamese (9%), Turkish (9%) and Moroccan (6%) (Jaddoe et al., 2006). The education level and household income were higher in the study cohort than in the study area, suggesting a slight selection towards higher SES (Jaddoe et al., 2008). However, the educational distribution in Generation R was similar to that in the Netherlands; in our sample 55.4% of mothers had a high level of education, this was 53.5% in the women in the Netherlands (reference year: 2002) (Statistics Netherlands, 2004).

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, and written informed consent was obtained from all adult participants.

In total, there were 8,976 children whose mothers were enrolled in the Generation R Study during pregnancy. Stress information was obtained by postal questionnaires at different time points during pregnancy. Most of the information on stress was collected when the mothers were 20-25 weeks pregnant. Mothers who did not reply to this questionnaire were excluded, as were those with information available on only one out of the four stress indicators (see Statistical Analysis). This left 6,812 mothers with available information on prenatal stress exposure at baseline. As our study was conducted in a multi-ethnic sample we examined measurement invariance of the stress construct across national origin groups. Due to lack of measurement invariance (see Statistical Analyses), mothers without information on their national origin could not be included in our study (n=138), as national origin-specific analyses could not be performed. In total, 6674 participants had information available at baseline for stress exposure and national origin. Non-verbal intelligence was assessed when children were 6 years old. Children who did not participate in this follow-up data collection wave (n= 2,168, 32.5% lost to follow-up) were excluded. Of the 4506 participants with available information at follow-up, children who had no complete IQ score and were, thus, set to 50, were not included as this typically represents invalid IQ scores (n=14). Children who had an IQ score ≤ 70

(n= 92) were included only in sensitivity analyses because these scores often indicate poor compliance during the IQ test. Children whose mothers were from non-western American countries (n=50) or Asian other than Indonesian (n=99) were not included as the sample size of these groups was smaller than the number of parameters in our models. This left 4,251 mother-child pairs for analyses (see Figure 1).

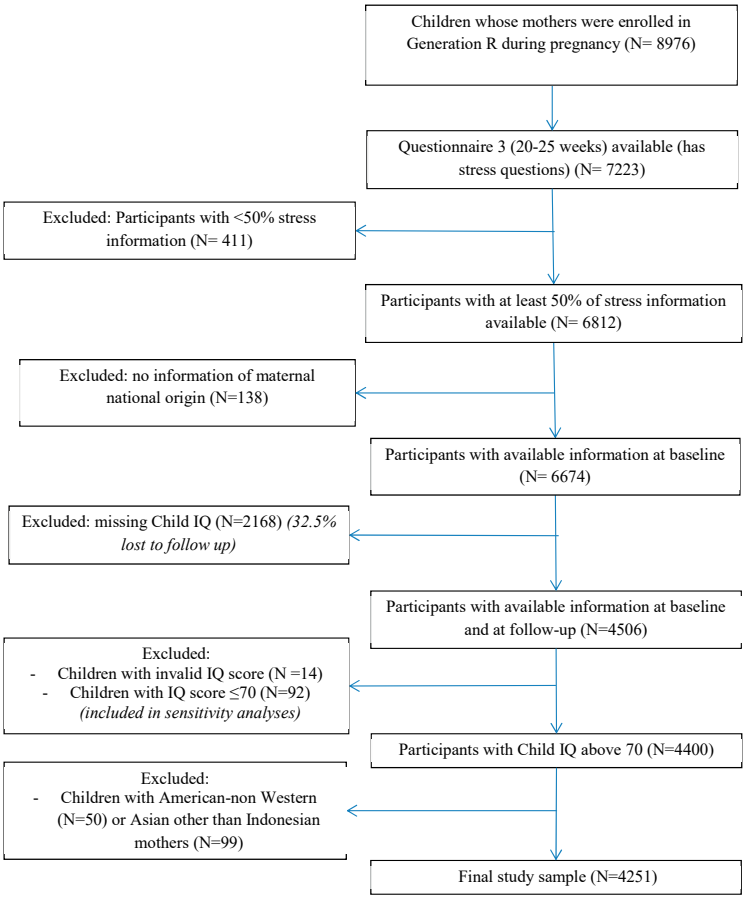

Figure 1. Flowchart of sample selection

Sample characteristics

Characteristics of the study sample are presented in Table 1 for the six main national origin groups: Dutch (n=2,567), Non-Dutch Western (n=380), Caribbean (n=426), Moroccan/Turkish (n=532), African (n=197), and Indonesian (n=149) (for more details see *Covariates* section). The majority of the mothers were of Dutch origin (60.0%) and the mean ages at enrollment ranged from 27.9 to 32.7 years. Most of the Dutch mothers had college or higher education (67.0%), while in the Caribbean, Moroccan and Turkish, and African mothers this percentage was below 30%. The average maternal IQ was 100.9

Table 1
Parental and Child Characteristics

Characteristics	Dutch (n=2567)	Non-Dutch Western (n=380)	Caribbean (n=426)	Moroccan/ Turkish (n=532)	African (n=197)	Indonesian (n =149)
	mean (SD) or %	**mean (SD) or %**	**mean (SD) or %**	**mean (SD) or %**	**mean (SD) or %**	**mean (SD) or %**
Maternal						
Age, years	31.7 (4.2)	31.2 (4.5)	28.5 (5.8)	27.9 (5.1)	28.4 (5.8)	32.7 (4.9)
Education, %						
Primary	0.9	3.5	5.9	20.4	14.3	0.7
Secondary	32.1	31.9	65.1	58.4	63.8	28.2
Higher	67.0	64.6	29.0	21.2	21.9	71.1
Smoking in pregnancy, %	13.9	14.7	17.1	21.2	15.7	14.1
Alcohol frequently in pregnancy, %	12.5	11.9	2.0	0.0	4.8	13.1
Maternal IQ score	100.9 (12.6)	98.5 (13.9)	90.2 (14.0)	86.0 (14.4)	85.4 (15.3)	101.6 (13.5)
Family income, %						
<1200 €	4.6	8.7	38.7	43.8	56.0	9.1
1200-2000 €	13.8	22.2	22.3	37.8	22.2	8.8
>2000 €	81.6	69.1	39.0	18.4	21.8	82.1
Paternal						
Education, %						
Primary	2.3	5.3	9.9	24.0	26.0	0.0
Secondary	32.1	37.6	66.1	50.2	51.7	28.0
Higher	65.6	57.1	24.0	25.8	22.3	72.0
Child						
Age, years	6.1 (0.4)	6.1 (0.4)	6.3 (0.7)	6.3 (0.6)	6.3 (0.6)	6.1 (0.5)
Child IQ score	105.3 (13.7)	101.6 (13.7)	96.7 (12.4)	96.2 (11.9)	97.4 (13.1)	105.2 (12.3)
Gender, % girls	51.4	55.5	50.0	49.2	51.8	51.7
Birthweight, grams	3472.5 (565.7)	3420.4 (565.8)	3203.5 (558.5)	3440.9 (502.0)	3268.3 (563.0)	3468.5 (594.1)

Note. The characteristics were measured in the imputed dataset (n= 4251).

(SD=12.6) in the Dutch group, 101.6 (SD=13.5) in the Indonesian, 98.5 (SD= 13.9) in the non-Dutch Western, 90.2 (SD= 14) in the Caribbean, 86.0 (SD= 14.4) in the Moroccan/ Turkish, and 85.4 (SD= 15.3) in the African group.

In our study 51.4% of the children were girls, and the average child non-verbal IQ score was 105.3 (SD=13.7) in the Dutch children, 105.2 (SD=12.3) in the Indonesian, 101.6 (SD= 13.7) in the non-Dutch Western, 96.7 (SD= 12.4) in the Caribbean, 96.2 (SD= 11.9) in the Moroccan/Turkish and 97.4 (SD= 13.1) in the African children (Table 1).

Measures

Prenatal Maternal Stress Exposure

To examine the association between a broadly operationalized prenatal stress measure and offspring IQ at age 6 years, we used a prenatal maternal stress exposure construct developed by Cecil et al. (2014) that has previously been implemented with good model fit using Generation R data (Rijlaarsdam et al., 2016). This stress construct is based on maternal reports during pregnancy in relation to four stress domains, accounting for different manifestations of stress: *life stress* (stressful life events such as illness, work related problems, pregnancy related anxiety), *contextual stress* (e.g., major financial difficulties, housing conditions), *personal stress* (e.g., psychiatric symptoms, criminal involvement) and *interpersonal stress* (e.g., family functioning, difficulties with others). For each stress domain, item scores were summed and divided by the number of completed items. Thus, higher scores represent greater stress exposure. For the present study, we adapted the stress construct by excluding maternal education and substance use from the stress domain variables. Although related to stress experience, maternal education and substance use have been shown to affect offspring neurobehavioral development mostly by pathways independent of maternal prenatal stress (Hanscombe et al., 2012; Olds et al., 1994; Olney et al., 2002). All stress domains were positively correlated (all $p<0.001$) (Table 2) (see Supplementary Material for full item and instruments descriptions (Supplementary Table 1)).

Child cognition

Child intelligence was measured in the research center by trained research assistants with a non-verbal IQ test when children were 5 to 7 years old. A non-verbal test was selected that minimized the potential bias related to the Dutch language abilities among children of non-Dutch origin. We administered two subtests of a validated Dutch nonverbal intelligence test: Snijders-Oomen Niet-verbale intelligentie test, 2.5-7- revisie (SON-R 2.5-7) (Tellegen et al., 1998). The subtests were 'Mosaics', that evaluates spatial insight, and 'Categories', that examines abstract reasoning abilities. The average alpha reliability of the total score of the SON-R 2.5-7 was of 0.90 (Tellegen et al., 1998) and

Table 2
Correlations between the prenatal stress domains

		Correlation matrix			
	Mean (SD)	Life stress	Contextual stress	Personal Stress	Interpersonal Stress
Stress domains					
Life stress	1.85 (1.5)	-			
Contextual stress	1.06 (1.4)	0.36	-		
Personal Stress	0.13 (0.4)	0.33	0.37	-	
Interpersonal Stress	1.90 (2.6)	0.33	0.42	0.42	-

Note. Correlation significant at the 0.01 level (2-tailed). All $p < 0.001$. n=4251

the reliabilities for the subtest scores that we used were 0.73 and 0.71 for Mosaics and Categories, respectively. The correlation between scores derived from the two applied subsets and the scores form the complete test was 0.86 (Tellegen, personal communication, 7 March 2011). The raw scores were transformed into non-verbal IQ scores, using age-specific normal values based on the exact child age (Tellegen et al., 1998).

The non-verbal IQ scores were normally distributed, ranging from 71 to 147 with a mean of 102.6 and a standard deviation (SD) of 13.8 in our study sample (Supplementary Figure 1). During the assessment, research assistants rated the child's motivation, collaboration and understanding of instructions (Basten et al., 2014).

Covariates

The variables selected as covariates were maternal IQ score, maternal and paternal educational level, family income, maternal alcohol consumption and smoking during pregnancy. We selected the confounders based on determinants of child cognitive development, the existing literature on the association between maternal prenatal stress and child intelligence (Eriksen et al., 2013; Henrichs et al., 2011; Tse et al., 2010), and by change-in-estimate criteria (cut-off of 5%). Maternal educational level, maternal alcohol consumption and smoking during pregnancy, family income and paternal education level were assessed with questionnaires during pregnancy. Educational level was indicated by the highest completed education, and was classified in primary, secondary and higher education. Smoking during pregnancy was collected at enrollment and during mid and late pregnancy and was categorized as non-smoking during pregnancy, smoking until pregnancy was known and continued smoking throughout pregnancy. Family income, defined by the total net monthly income of the household, was classified as below social security level (less than 1200 €), low income (1200 to 2000 €) and modal to high income (more than 2000 €). Maternal intelligence was measured at the same time when child IQ data was collected, when the mother accompanied the 6-year old child to the research center, with the set I of the Raven's Advanced Progressive Matrices

Test (Raven, 1962). This test has been shown to be a valid and reliable short form of the Raven's Progressive Matrices test to assess non-verbal cognition (Chiesi et al., 2012; *Raven´s Advanced Progressive Matrices [APM]. Evidence of Reliability and Validity*, 2007) and the non-verbal aspect of the test minimizes the impact of the language abilities in the non-Dutch mothers in our study.

Maternal national origin

Information on maternal national origin was collected by questionnaires during pregnancy and was defined according to Statistics Netherlands (2004). The national origin classification was based on the country of birth of the parents of the participant. If one of the parents was born abroad, the participant was considered to be of non-Dutch origin. If both parents were born abroad, the country of birth of the participant's mother defined the participant's origin. Maternal national origin was initially categorized as Dutch, non-Dutch Western, and non-Western. The non-Dutch Western mothers came from European, Oceanian and Western American countries. The non-Western group included four subgroups: Caribbean (Dutch Antillean and Surinamese), Moroccan/Turkish (Moroccan and Turkish), African (Cape Verdian and other African), and Indonesian mothers. In the minority groups, a questionnaire on self-reported Dutch language ability was administered during pregnancy. Dutch language ability was defined as a composite of three questions that referred to reading, writing and speaking abilities. The sense of belonging to the Dutch culture was assessed during pregnancy based on the extent to which the participant agreed with the following statement: "I feel part of the Dutch culture". Following recent evidence that supports the predictive validity of single-items measures (Bowling, 2005), these items (i.e. language abilities and feeling part of the Dutch culture) were used as proxy measures for maternal acculturation. Higher scores indicated higher levels of acculturation. The distribution of these variables in the various national origin groups is described in the Supplementary Table 2.

Statistical Analysis

Measurement model

A reflective, standardized latent construct of maternal prenatal stress was estimated in R (Lavaan Package developmental version 0.6-1.1141) using the previously described four stress domains (life stress, contextual stress, personal stress and interpersonal stress) as indicators. Internal reliability of the stress domains and of the latent construct of maternal prenatal stress was assessed with confirmatory factor analyses (see Rijlaarsdam et al., 2016). The latent construct showed good model fit as judged with the comparative fit index (CFI, acceptable fit ≥ 0.90 (McDonald & Ho, 2002)).

An important requirement for a meaningful interpretation of the analysis with a latent construct is the measurement invariance of the construct across relevant subgroups in the population (i.e. the meaning of the concept of stress is the same across members of relevant subgroups) (Milfont & Fischer, 2010). We performed measurement invariance tests to examine whether the maternal stress construct was similar across child sex and maternal national origin subgroups as our cohort is a multi-ethnic sample. The invariance of the latent construct was evaluated with a series of hierarchically nested multi-group models in which constraints of the loadings, intercepts and variances of the latent variable were progressively implemented. To decide whether a more restricted model fitted as well as a less restricted model, we used the delta comparative fit index (CFI) cutoff of <0.01 (Hirschfeld & von Brachel, 2014), which is robust for testing the between-group invariance of latent variable models (Cheung & Rensvold, 2002). Measurement invariance was tested across the groups of maternal national origin. We aimed to define broad national origin groups in whom the stress construct was invariant. First, measurement invariance tests were performed across the three main maternal national origin groups (i.e. Dutch, Non-Dutch Western, and non-Western). Due to lack of invariance across these groups (i.e. lack of equivalent psychometric properties) (see Results section), we could not validly examine global prenatal stress in an un-stratified sample. Therefore, the subsequent analyses were performed in more narrowly defined groups, organized by the national origins of the participants and socioeconomic characteristics shared by specific national origin groups (Odé, 2002). The main analyses were performed in six subgroups: Dutch, Non-Dutch Western, Caribbean, Moroccan/Turkish, African and Indonesian (see factor loadings for the measurement model of global prenatal maternal stress for the different groups in Table 3).

Table 3
Standardized factor loading estimates (and standard errors) for the Global Prenatal Maternal Stress in the measurement model for the different national origin groups.

	Factor Loadings					
	Dutch	**Non-Dutch Western**	**Caribbean**	**Moroccan/ Turkish**	**African**	**Indonesian**
	n=2567	n=380	n=426	n=532	n=197	n=149
Indicators	Estimate (SE)	Estimate (SE)	Estimate (SE)	Estimate (SE)	Estimate (SE)	Estimate (SE)
Life Stress	0.44 (0.03)	0.54 (0.08)	0.52 (0.07)	0.57 (0.06)	0.56 (0.08)	0.67 (0.20)
Contextual Stress	0.55 (0.04)	0.57 (0.07)	0.61 (0.06)	0.48 (0.05)	0.79 (0.07)	0.40 (0.15)
Personal Stress	0.48 (0.06)	0.57 (0.12)	0.59 (0.07)	0.70 (0.05)	0.60 (0.08)	0.27 (0.18)
Interpersonal Stress	0.59 (0.04)	0.62 (0.08)	0.62 (0.07)	0.56 (0.05)	0.60 (0.08)	0.49 (0.18)

Structural Path Analysis

The construction of the measurement model and the analyses of the association between the latent construct of maternal prenatal stress and child cognition were simultaneously performed using structural equation modelling in R (Lavaan Package). Analyses (Path model: Figure 2) were conducted in the following subgroups: Dutch, Non-Dutch Western, Caribbean, Moroccan/Turkish, African and Indonesian. The models were estimated with the robust maximum likelihood estimator (Huber-White standard errors) and the latent variable was scaled by standardization of the latent variable variance to 1. The path coefficients represent the change in the predicted child IQ score per standard deviation increase in maternal prenatal stress.

Confounders were initially selected based on existing literature and then by change-in-estimate criteria (cut-off of 5%) (Mickey & Greenland, 1989), that is, evaluating the change in the estimate of the association between maternal stress and child intelligence when adding each confounder to the univariate regression. The confounders selected were maternal IQ, maternal education, paternal education, family income, maternal smoking during pregnancy and alcohol consumption during pregnancy. Four models of the association between maternal stress during pregnancy and child intelligence were constructed with a progressive inclusion of covariates to show the impact of the adjustment for groups of key confounders. Adjustment for confounders was applied to the latent construct and to the prenatal stress - child IQ path. The goodness of fit of these progressively adjusted models was compared with the Bayesian information criterion (BIC) and Akaike's information criterion (AIC). A lower value for AIC and BIC indicates a better fit (Jouni, 2004).

Sensitivity Analyses

In additional analyses, we explored the association between the prenatal stress domains included in the latent construct and child IQ using multiple linear regression analyses. As in the overall model, the analyses were performed in each national origin group and with similar adjustment for confounders. The regression estimates are presented for the standardized stress indicators.

We further examined the associations between the different prenatal stress measures and child IQ by linear regression analyses; every stress measure included in our stress latent construct was studied (For more information see Supplementary table 1). These analyses were performed in the complete un-stratified sample and also separately for each national origin group, controlling for maternal IQ, SES-related factors and substance use. We further adjusted the associations in the complete sample for national origin groups in a second model. Analyses were not performed in a subgroup of stress variables with less than 10 exposed participants.

As SES has been shown to moderate the amount of variance in IQ explained by the shared environment (Turkheimer et al., 2003), we explored the role of SES as a moderator in the association of prenatal stress and child IQ. To this aim, we calculated the principal component of the different SES measures (i.e. maternal and paternal education and household income). Following our main analyses, we ran an additional model adding the SES-principal component as predictor and its interaction with the stress latent construct. The stress latent construct and the interaction term were orthogonalized (i.e. their covariance was fixed to 0).

To better represent the variation of low IQ scores in the general population, we also tested if the association between prenatal stress and child IQ changed if children with IQ scores below 70 (n=92), which are considered less valid (Mackenzie & Wonders, 2016), were included. We also used a more conservative approach in a subsequent sensitivity analysis and included only children (n=3702) who had good motivation, collaboration, and understanding of instructions during the IQ test.

Missing data

The sample (n= 4,251) had complete information for child IQ scores, maternal national origin and for at least two of the four stress indicators. All missing value frequencies per variable were below 15% (maximum: Contextual Stress = 11.3%). Multiple imputation was performed for the missing values of the stress indicators and the confounders in the statistical software of R (MICE package version 2.46), using 40 imputations. The semTools package (semTools version 0.4-15.910) was used to pool the estimates.

The acculturation variables were included in a post hoc analysis. These variables were only available for national origin minorities. The maximum percentage of missing values was 21.2% for the variable "Feeling part of the Dutch culture" and 14.5% for Language abilities in the Moroccan/Turkish mothers.

Non-response analysis

Differences in baseline characteristics between children included in our study sample and those who were not included were evaluated using Chi-square tests for the categorical and independent t-tests for the continuous variables (See also the baseline characteristics of children lost to follow-up in Supplementary table 3). The non-response analyses showed that mothers of children who were not included were younger (mean maternal age= 28.7) and less educated (13.4% of these mothers had only primary education) than mothers of children in the study (mean age = 30.7, primary education= 4.6%). Also, excluded mothers were more likely to smoke throughout pregnancy (19.0%) than mothers of children in our study sample (15.3%).

RESULTS

Measurement model

Analyses of measurement invariance of the stress latent construct showed strict invariance when grouping participants by child sex (delta CFI< 0.01), but lack of invariance across the broad maternal national origin groups (i.e. Dutch, non-Dutch Western, and non-Western) (Supplementary Table 4) and across the national origin subgroups of the non-Western mothers (Caribbean, Moroccan/Turkish, African and Indonesian) (n= 1,304) (Supplementary Table 5). However, there was strong measurement invariance within the Moroccan/Turkish, the Caribbean (Surinam and Dutch Antilles) and the African (Africa other than Moroccan, and Cape Verde) subgroups (Supplementary Table 6, 7 and 8). As there was lack of invariance across the broad national origin groupings but strong measurement invariance in more narrowly defined national origin groups, the association of maternal prenatal stress and child IQ was evaluated in six groups separately: Dutch, Non-Dutch Western, Caribbean, Moroccan/Turkish, African, and Indonesian.

The measurement model and the structural equation model are depicted in Figure 2. The loadings of the latent stress construct on each stress indicator variable ranged from 0.27 (personal stress in the Indonesian mothers) to 0.79 (contextual stress in the African mothers) in the six national origin groups (Table 3).

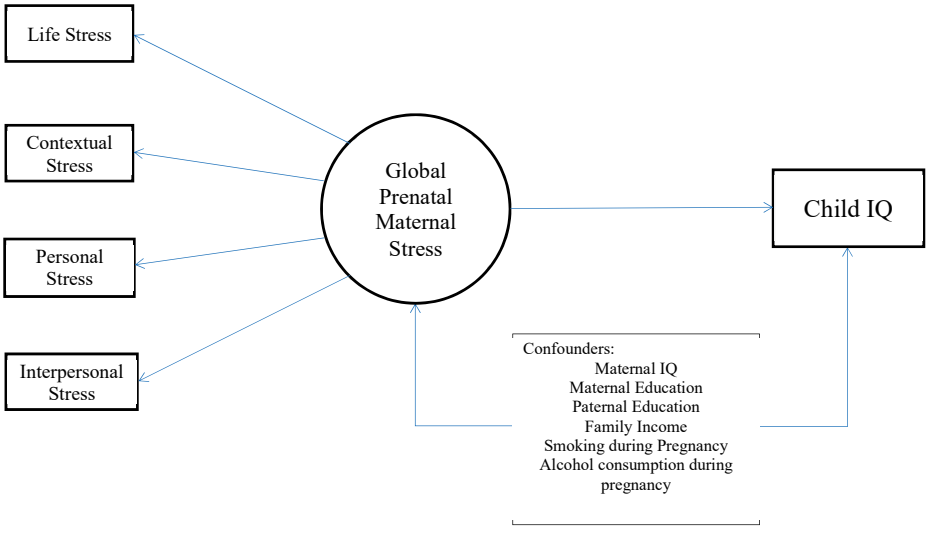

Figure 2. Path Model of the Structural Equation Model for the association between Global Prenatal Maternal Stress and Child IQ scores.

Maternal prenatal stress and child IQ

Table 4 shows the association between the latent construct of maternal prenatal stress and child IQ in the six national origin groups (Dutch, non-Dutch Western, Caribbean, Moroccan/Turkish, African, and Indonesian). Results are shown with the stepwise confounder adjustment and presented as model 1 (unadjusted model) to model 4 (fully adjusted model) (Table 4).

In the Dutch population, a one-standard-deviation increase in the levels of maternal stress was associated with 1.57 (SE=0.36) points lower child IQ score in the initial analyses. After adjusting for maternal intelligence, the association was attenuated (B = -1.13 points, SE = 0.35) and finally disappeared in model 3, after additional adjustment for the socioeconomic indicators, i.e. maternal and paternal education and family income (B = -0.30 points, SE=0.38, p-value= 0.43). A similar stepwise reduction of the association of prenatal maternal stress and child intelligence was observed in the Caribbean group, in

Table 4
Association of Global Maternal Prenatal Stress (latent variable) with Child IQ

		Child IQ							
		Model 1		Model 2		Model 3		Model 4	
National origin group	n	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*
Dutch	2567	-1.57 (0.36)	<0.001	-1.13 (0.35)	0.001	-0.30 (0.38)	0.431	-0.19 (0.39)	0.631
Non-Dutch Western	380	-0.25 (1.01)	0.805	0.09 (0.97)	0.929	0.62 (1.01)	0.539	0.51 (1.01)	0.613
Non-Western									
Caribbean	426	-1.58 (0.67)	0.019	-1.45 (0.66)	0.028	-0.61 (0.72)	0.398	-0.43 (0.74)	0.561
Moroccan/Turkish	532	-1.83 (0.65)	0.005	-1.73 (0.63)	0.006	-1.44 (0.64)	0.024	-1.53 (0.63)	0.015
African	197	0.24 (1.07)	0.820	0.25 (1.08)	0.820	0.48 (1.14)	0.674	0.06 (1.22)	0.960
Indonesian	149	-0.22 (1.74)	0.899	-0.49 (2.01)	0.809	-2.01 (1.83)	0.271	-1.97 (3.54)	0.577

Note. Models were constructed using SEM (Lavaan package). Values are regression coefficients, standard errors and *p* values. The estimates are based on the standardized latent factor (Global Stress). A progressive adjustment was applied to the latent factor and to the structural path as listed below. All models converged in at least 38 datasets. In the Indonesian group, model 2 and 4 had pooled negative variance for the indicator of contextual stress which probably reflects a relatively low sample size given the numbers of parameters. n=4251
Model 1. Without adjustment
Model 2. Model 1 + Maternal IQ
Model 3. Model 2 + Maternal education + Paternal education + Family income
Model 4. Model 3 +Maternal smoking during pregnancy + Maternal alcohol consumption during pregnancy

whom the association disappeared in the third model, after adjustment for SES (B = -0.61 points, SE=0.72, p-value= 0.40).

No association between maternal stress during pregnancy and child intelligence was observed in the unadjusted model in the Non-Dutch Western, the African and the Indonesian groups. In contrast, a one-standard-deviation increase in the levels of prenatal stress in the Moroccan and Turkish mothers was related to 1.83 point lower child IQ in the initial model, and this association was only slightly attenuated after complete adjustment for potential confounders (model 4: B = -1.53 points, SE=0.63).

The goodness of fit of these progressively adjusted models was evaluated with the AIC and BIC for each national origin group (Supplementary table 9). Both criteria indicated in general a better fit of the models with additional adjustment for SES-related factors and substance use (i.e. model 3 and 4).

We performed exploratory regression analyses between each of the maternal stress indicators and child IQ in the different national origin groups. In the Dutch population, no association was found between any of the stress indicators (i.e. life stress, contextual stress, personal stress and interpersonal stress) and child IQ after controlling for confounders (Table 5). Likewise, in the Moroccan/Turkish group, only suggestive associations between the maternal stress indicators and child IQ were observed after adjustment (Table 6). The stress domains were not related to child IQ in the other national origin groups, except for one of four domain measures in the Indonesians, in whom one standard deviation higher contextual stress in the mother was related to a 3-point lower offspring IQ (SE=1.36, p-value=0.02) (Supplementary table 10).

Table 5

Association between Indicators of Maternal Prenatal Stress and Child IQ score in the Dutch group

	Child IQ							
	Model 1		Model 2		Model 3		Model 4	
	b (se)	*P*	b (se)	*P*	b (se)	*P*	b (se)	*P*
Stress variables								
Life stress	-0.39 (0.32)	0.22	-0.19 (0.31)	0.54	0.09 (0.32)	0.79	0.16 (0.33)	0.61
Contextual stress	-1.10 (0.36)	0.002	-0.72 (0.34)	0.03	0.01 (0.39)	0.98	0.10 (0.39)	0.81
Personal Stress	-1.19 (0.39)	0.002	-0.82 (0.39)	0.04	-0.30 (0.40)	0.46	-0.19 (0.41)	0.64
Interpersonal Stress	-1.46 (0.36)	<0.001	-1.19 (0.35)	0.001	-0.57 (0.37)	0.13	-0.53 (0.37)	0.15

Note. Models were constructed using multiple linear regression (SEM function, MLR estimator). Values are regression coefficients, standard errors and *p* values. The stress variables were standardized. n= 2567.

Model 1. Without adjustment

Model 2. Model 1 + Maternal IQ

Model 3. Model 2 + Maternal education + Paternal education + Family income

Model 4. Model 3 + Maternal smoking during pregnancy + Maternal alcohol consumption during pregnancy

Table 6

Association between Indicators of Maternal Prenatal Stress and Child IQ score in the Moroccan/ Turkish group

	Child IQ							
	Model 1		Model 2		Model 3		Model 4	
	b (se)	*P*	b (se)	*P*	b (se)	*P*	b (se)	*P*
Stress variables								
Life stress	-0.86 (0.44)	0.05	-0.91 (0.43)	0.04	-0.77 (0.45)	0.09	-0.82 (0.44)	0.06
Contextual stress	-0.93 (0.46)	0.04	-0.97 (0.46)	0.03	-0.64 (0.49)	0.19	-0.65 (0.49)	0.19
Personal Stress	-0.69 (0.42)	0.10	-0.67 (0.42)	0.11	-0.59 (0.44)	0.18	-0.62 (0.41)	0.13
Interpersonal Stress	-1.43 (0.51)	0.01	-1.16 (0.51)	0.02	-0.92 (0.53)	0.09	- 0.96 (0.51)	0.06

Note. Models were constructed using multiple linear regression (SEM function, MLR estimator). Values are regression coefficients, standard errors and *p* values. The stress variables were standardized. All models converged in at least 25 imputed datasets. n= 532.
Model 1. Without adjustment
Model 2. Model 1 + Maternal IQ
Model 3. Model 2 + Maternal education + Paternal education + Family income
Model 4. Model 3 + Maternal smoking during pregnancy + Maternal alcohol consumption during pregnancy

In order to test if the association between maternal prenatal stress and child intelligence in the Moroccan/Turkish group was explained by minority stress, we performed a post-hoc sensitivity analysis in this national origin group. We adjusted the association of maternal prenatal stress and child IQ in model 2 (i.e. model adjusted only for maternal IQ) for maternal acculturation variables (feeling part of the Dutch culture and language abilities). The association between maternal stress and child IQ in the Moroccan/Turkish participants remained essentially unchanged after this adjustment (B = -1.73 points, SE=0.63, p-value=0.01). Additionally, in separate linear regression models, we observed that acculturation was not associated with child IQ in the Moroccan/Turkish group. Better maternal language abilities were, however, related to less prenatal stress in the Moroccan/Turkish group. Also, this minority had the lowest level of self-reported acculturation in our sample (Supplementary table 2). The sensitivity analyses of the associations between the individual stress measures and child IQ are presented in Supplementary table 11. The analyses in the complete study sample demonstrate the independent contribution of national origin to the association of prenatal stress and child IQ. After additional adjustment for national origin groups (model 2), the associations between most stress variables and child IQ (already adjusted for maternal IQ, SES and substance use) were substantially reduced. Only family conflict and lack of satisfaction with obstetric care were related to lower child IQ after full adjustment for potential confounders. However, these results should be interpreted cautiously given the number of tests. We also explored the role of SES as a moderator in the association of the latent prenatal stress construct and child IQ; no significant interaction with SES on the association with IQ was observed (Supplementary table 12).

The analyses of the association between prenatal stress and IQ including children with an IQ below 70 are presented in Supplementary table 13. The associations did not change substantially, with one exception, in the Moroccan/Turkish group the association was attenuated in model 3 and was only borderline significant. When we included only children who showed good compliance during the IQ test the associations after full adjustment for confounders were essentially unchanged if compared with the primary analysis. In this latter sensitivity analysis the association between stress and child IQ in the Moroccan/Turkish group remained after adjustment for confounders (B = -1.64 points, SE=0.79, p-value=0.038), indicating possible information bias in the sensitivity analysis that included children with very low IQ.

DISCUSSION

In the Dutch group and in the Caribbean minority, no association between maternal self-reported prenatal stress and child IQ was observed after adjustment for maternal IQ and socioeconomic-related factors, contrary to our main hypothesis. No association was found, even before adjustment, between prenatal stress and child IQ in the non-Dutch Western, the African and the Indonesian groups. Only in the Moroccan and Turkish mother-child dyads, maternal prenatal stress predicted a lower child IQ score after adjustment for confounders.

Most of the previous prenatal stress research focused on psychiatric problems such as depressive symptoms (Tse et al., 2010), anxiety (Van den Bergh et al., 2005) or in generally perceived stress (Slykerman et al., 2005), and the studies that assessed more than one manifestation of stress usually investigated the association with child cognition in separate models (e.g., DiPietro et al., 2006). In contrast, we assessed maternal prenatal stress with a latent construct based on different life domains, capturing more indices of maternal stress during pregnancy. As stressful events tend to co-occur, the simultaneous assessment of various indices of prenatal stress is more relevant to public health than the single stress measurements (Felitti et al., 1998). Additionally, the latent construct allowed us to systematically examine the shared variance of the different stress manifestations (Tinsley & Tinsley, 1987) and to test whether the stress experience was similar across different groups in society (Milfont & Fischer, 2010). Previous studies on the relation between maternal stress and offspring cognition in school-age children reported mixed findings. Some observed a negative effect of stress while others did not find any association. These studies typically focused on specific stress measures, such as depressive symptoms, anxiety or stressful events. We do not intend to reconcile this conflicting evidence with the current study, but to extend the scientific knowledge with

a complementary approach: testing the association between a broad, cumulative stress construct and cognitive functioning of school-age children.

The adjustment of the association between maternal prenatal stress and child IQ for family income, maternal and paternal education and maternal alcohol consumption and smoking during pregnancy could be seen by some scholars as over-adjustment since these variables are life-style factors generally related to the concept of stress (Cohen et al., 2006; Hassanbeigi et al., 2013), thus the adjustment for these variables was performed in a late step and in separate models. These factors affect offspring mental development through multiple pathways other than maternal stress during pregnancy (Eriksen et al., 2013; Hanscombe et al., 2012; Olds et al., 1994; Olney et al., 2002). Parental education influences child IQ mainly by three pathways: first, education is highly correlated with IQ and thus the association of parental education and child IQ is strongly genetically determined. Second, parental education affects child cognitive development through parenting and upbringing. Third, low parental education can also be seen as a cause of stress, e.g., financial stress during pregnancy. Importantly, financial stress and other education-related stress indicators were accounted for by the indicator of contextual stress. Likewise, the consumption of alcohol and tobacco during pregnancy can be seen as a marker of stress. However, pregnant women in the Netherlands are not consistently advised to be totally abstinent. Consequently, alcohol consumption in our and other study samples is associated with indicators of well-being (Kelly et al., 2012). The inclusion of these socioeconomic status indicators and substance use in the stress construct would inappropriately broaden the stress definition, with implications for the etiological understanding and public health interventions.

In the offspring of the Dutch and Caribbean mothers, the association between maternal prenatal stress and child IQ was explained by maternal IQ and socioeconomic indicators (i.e. parental education and family income). Differences in the association between prenatal stress and child IQ across national origin groups may represent a difference in the understanding of stress and the influence of the transactive nature of the genetic cognitive potential and the postnatal environment in the prediction of offspring cognition. In the Moroccan/Turkish group, mothers had, on average, a lower IQ score than the mean in our sample (average maternal IQ score = 97.1) and the percentage of mothers with only primary education was the highest among all national origin groups. In contrast, the lack of association between prenatal stress and child IQ, even in the unadjusted model, in the Non-Dutch Western, the African and the Indonesian groups can best be explained by the lack of variance in child IQ accounted for by SES-related factors due to the homogeneity in social background.

Parental education level, as a proxy for "environmental quality" (i.e. financial resources and intellectual stimulation), is suggested to be a moderator for the heritability of IQ; typically, the IQ heritability is higher when the level of parental education is higher.

Also, Rowe et al. (1999) demonstrated that the proportion of variance in IQ explained by the shared environmental effects, such as socioeconomic status and family structure, is higher when the level of parental education was lower. Thus, in those national origin groups with high parental education the absence of an association between prenatal stress and IQ could be related to the low variance explained by the shared environment (such as a maternal stress). In contrast, the low education in the Moroccan and Turkish parents would imply that the IQ phenotype would be highly responsive to environmental variation. Additionally, the Moroccan and Turkish mothers may be sharing a sustained stressor, that would prolong maternal stress into the postnatal period and therefore influence cognitive development, through lower cognitive stimulation, parenting style, and the home environment (Guo & Harris, 2000; Jensen et al., 2014).

We examined the role of minority stress in the negative association between prenatal maternal stress and child IQ in the Moroccan and Turkish. More than 25% of the population of Rotterdam are immigrants, and most of them come from Surinam, Turkey or Morocco (Entzinger & Engbersen, 2014). The native majority in the Netherlands corresponds to a relatively affluent and financially equal population with a good quality of life (Eurostat Statistical Book, 2015), while the minorities encounter high levels of stress related to the pressure of integration and acculturation (Smith, 1985; 2005). The integration of minorities in the Netherlands remains particularly difficult for the immigrants coming from Morocco or Turkey (2005) as they display low levels of upward social mobility (Uitermark, 2003). We explored the influence of minority stress, as measured by the acculturation variables, on the association between maternal stress and child IQ. Among all minorities, the Moroccan and Turkish mothers had the lowest levels of self-reported acculturation. However, acculturation did not confound the association between maternal stress and child IQ in the Moroccan/Turkish sample. We cannot rule out that the low acculturation in the Moroccan and Turkish mothers, compared to other national origin groups, may indicate a vulnerability to stress, as postulated by Dyal and Dyal (1981). We were not able to test the interaction between maternal stress and acculturation as maternal prenatal stress was not invariant across national origin groups. Several other limitations must be also considered. First, the latent construct lacks direct measure and does not have inherent units (Ramlall, 2016). Therefore, the direct translation of our results to public health may be limited. However, it is difficult to imagine how a broad concept of stress could be directly measured. Second, it is possible that the latent, broad, stress construct approach obscures associations of specific stress measures, such as the association observed between the contextual stress domain and child IQ in the Indonesian group. However, an association found while examining various stress measures in different groups could be the result of multiple testing and thus reflect chance. Third, we observed a higher average IQ in the children compared to maternal IQ in all national origin groups. This may reflect the difference in the IQ measures used in children and

mothers, and thus absolute IQ mother-child differences cannot be evaluated. Additionally, although a valid assessment of performance, maternal IQ is an imperfect measure of child genetic potential, as the offspring IQ is also determined by postnatal factors such as schooling (Brinch & Galloway, 2012) and by paternal IQ. In order to account for the child genetic potential we could only adjust our analyses for maternal and not for paternal IQ. Fourth, the mothers who did not respond were younger and less educated, characteristics that may suggest a higher risk of experiencing higher levels of stress. The absence of these participants in our study may affect the generalizability of our results. Fifth, we grouped participants in national origin groups according to the presence of stress-related environmental factors, such as their socioeconomic background (Odé, 2002). Yet, genetic and cultural differences across and even within countries remain. However, the observed measurement invariance of the stress construct in our sample supported our grouping, suggesting that these national origin groups share a similar understanding of the concept of stress (Milfont & Fischer, 2010). Sixth, the Indonesian group had a small sample size relative to the number of parameters in the model, limiting the precision of results in this group.

The present study contributes to a better understanding of the association between maternal stress during pregnancy and child intelligence in the general population. We did not find support for the model predicting prenatal stress effects on later cognitive development in the majority of our study sample. We showed that any observed association was mostly explained by maternal IQ and socioeconomic status indicators, except in the least acculturated national origin group. Most likely, postnatal factors, such as social disadvantage, sustained maternal stress, and ethnic-related stressors, may play a role in the offspring cognitive development of less acculturated minorities.

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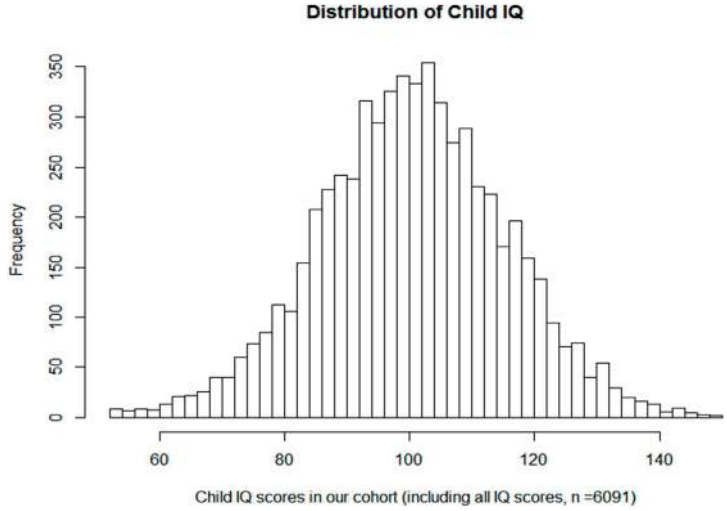
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Distribution of Child IQ scores (including all IQ scores)

Supplementary Table 1. Stress domains and Instruments used

Stress Domain	Instrument	General description of the instrument	Assessment time point	Selected Items	Reference
Life stress	Social Readjustment Rating Scale (SRRS)	We applied a Dutch-adapted version (10 items) of the SRRS questionnaire to assess the ocurrence of repeated stressful life events in the preceding 12 months (yes-no questions).	20-25 weeks gestation (ges.)	Death of partner or child	Holmes, T. H. & Rahe, R. H. (1967). The social readjustment rating scale. Journal of Psychosomatic Research, 11(2), 213-221.
				Death of a friend or relative	
				Serius illness of child, partner or relative	
				Job loss	
				Residence change	
	12- Item Short Form Health Survey (SF-12)	The SF-12 is a self-report health-related quality of life questionnaire. We applied an adapted version with 15 items, varying in the number of possible answers.	20-25 weeks ges.	Personal illness (moderate or poor health)	Ware, J. E., Kosinski, M., & Keller, S. D. (1996). A 12-Item short-form health survey: Construction of scales and preliminary tests of reliability and validity. Medical Care, 34(3), 220–233.
	Long Lasting Difficulties Questionnaire	Mothers reported whether they had had financial, health or social problems. These long-lasting difficulties were rated in a 5-point scale using a 12-items questionnaire that assesses problematic situations in the preceding year. We included the questions of problems at work or school in the Life Stress domain.	20-25 weeks ges.	Problems at work or school	Hendriks, A. A. J., Ormel, J. and van de Willige, G. (1990). Long lasting difficulties measured with a self-assessment questionnaire and semistructured interview: A theoretical and empirical comparison [in Dutch]. Gedrag en Gezondheid, 18: 273–283.

Supplementary Table 1. Stress domains and Instruments used (*continued*)

Stress Domain	Instrument	General description of the instrument	Assessment time point	Selected Items	Reference
	General Pregnancy Questions - Generation R	Mothers reported general pregnancy information during different time points of their pregnancy (8 items with different number of possible answers).	12-20 weeks ges.	Unplanned pregnancy	Jaddoe, V. W., van Duijn, C. M., van der Heijden, A. J., Mackenbach, J. P., Moll, H. A., Steegers, E. A., ... & Hofman, A. (2008). The Generation R Study: design and cohort update until the age of 4 years. European journal of epidemiology, 23(12), 801.
			20-25 weeks ges.	Vaginal bleeding in preceding two months	
			30 weeks ges.	Chromosomal abnormalities tests (e.g. Chorionic villus sampling)	
				Insatisfaction with obstetric care	
				Admission to a hospital for more than 24 hours	
	General Life Events Questions - Generation R	Mothers reported whether they had been victims of a violent offense in the preceding 12 months (yes-no question).	20-25 weeks ges.	Victim of robbery, theft, physical abuse or rape	Jaddoe, V. W., van Duijn, C. M., van der Heijden, A. J., Mackenbach, J. P., Moll, H. A., Steegers, E. A., ... & Hofman, A. (2008). The Generation R Study: design and cohort update until the age of 4 years. European journal of epidemiology, 23(12), 801.
	Pregnancy Outcome Questionnaire (POQ)	We applied a 13-items Dutch adapted version of the Pregnancy Outcome Questionnaire to evaluate anxiety concerning the pregnancy and the baby. Items were rated on a 4-point Likert-Scale. We included the questions: “I am worried about the health of the baby” and “I worry about whether the pregnancy will go well or not” in the Life Stress domain.	12-20 weeks ges.	Worry about the health of the baby	Theut, S. K., Pedersen, F. A., Zaslow, M. J., & Rabinovich, B. A. (1988). Pregnancy subsequent to perinatal loss: Parental anxiety and depression. Journal of the American Academy of Child & Adolescent Psychiatry, 27(3), 289-292.
				Worry about the pregnancy	

Supplementary Table 1. Stress domains and Instruments used (*continued*)

Stress Domain	Instrument	General description of the instrument	Assessment time point	Selected Items	Reference
Contextual Stress	General Sociodemographic Questions - Generation R	Mothers reported whether their house had major appliances (3 items, yes-no questions), whether their house had defects (3 items, yes-questions) and whether they had financial difficulties in the preceding 12 months (1 item, 3-point Likert-Scale)	30 weeks ges.	Lack of housing major appliances (heating, washing machine, refrigerator)	Jaddoe, V. W., van Duijn, C. M., van der Heijden, A. J., Mackenbach, J. P., Moll, H. A., Steegers, E. A., ... & Hofman, A. (2008). The Generation R Study: design and cohort update until the age of 4 years. European journal of epidemiology, 23(12), 801.
				Housing defects (such as draughts, windows with condensations or dampness)	
				Financial difficulties (e.g. difficulties in paying rent)	
	Long Lasting Difficulties Questionnaire	See description in the Life Stress Domain. The items related to housing adequacy and financial problems were included in the Contextual Stress domain.	20-25 weeks ges.	House inadequacy (e.g., too small, lack of privacy)	-
				Major financial problem (e.g. income not sufficient)	
	Social Readjustment Rating Scale (SRRS)	See description in the Life Stress Domain.	20-25 weeks ges.	Downturn in financial situation	-
Personal Stress	General questions about public order offenses - Generation R	We applied a self-report 20-items questionnaire adapted by Generation R from Jeugddelinquentie: Risico's en bescherming [Delinquency in youth: Risk and protective factors], to assess law violations and offenses. Items were rated in a 5-point Likert-Scale. Questions about public order offenses, interpersonal violence and the existence of a criminal record in the Personal Stress domain.	20-25 weeks ges.	Having a criminal record	Laan, A.M. van der, Blom, M., Verwers, C., Essers, A.A.M (2006). Jeugddelinquentie: Risico's en bescherming ; bevindingen uit de WODC Monitor Zelfgerapporteerde Jeugdcriminaliteit 2005. [Delinquency in youth: Risk and protective factors].Den Haag, the Netherlands: Boom Juridische uitgevers.
				Violent interpersonal offenses	
				Public order offenses	

Supplementary Table 1. Stress domains and Instruments used (*continued*)

Stress Domain	Instrument	General description of the instrument	Assessment time point	Selected Items	Reference
	General Sociodemographic Questions - Generation R	We assessed maternal age (open question) to identify the mothers who were 18 years old or younger.	enrollment	Early parenthood (age mother < 19 years)	Jaddoe, V. W., van Duijn, C. M., van der Heijden, A. J., Mackenbach, J. P., Moll, H. A., Steegers, E. A., ... & Hofman, A. (2008). The Generation R Study: design and cohort update until the age of 4 years. European journal of epidemiology, 23(12), 801.
	Brief Symptom Inventory (BSI)	The BSI is a 53-item self-report questionnaire that assesses psychiatric problems in the preceding 7 days. The items are rated on a 5-point Likert-scale.	20-25 weeks ges.	Maternal psychopathology: Global BSI score	Derogatis, L. R. (1993). Brief Symptom Inventory (BSI); Administration, scoring, and procedures manual. Pearson.
Interpersonal Stress	Long Lasting Difficulties Questionnaire	See description in the Life Stress Domain. Questions about interpersonal problems were included in the Interpersonal Stress domain.	20-25 weeks ges.	Arguments with partner	-
				Arguments with family or friends	
				Difficulties in contact with others	
	General Sociodemographic Questions - Generation R	Mothers reported their marital status (multiple choice question) and the number of people living in their house (open question).	12-20 weeks ges.	Marital status	Jaddoe, V. W., van Duijn, C. M., van der Heijden, A. J., Mackenbach, J. P., Moll, H. A., Steegers, E. A., ... & Hofman, A. (2008). The Generation R Study: design and cohort update until the age of 4 years. European journal of epidemiology, 23(12), 801.
			20-25 weeks ges.	Large family size	

Supplementary Table 1. Stress domains and Instruments used (*continued*)

Stress Domain	Instrument	General description of the instrument	Assessment time point	Selected Items	Reference
	Family Assessment Device (FAD)	The FAD is a self-report questionnaire that evaluates several dimensions of family functioning. In this study, we applied the General Family functioning subscale (12 items rated on a 4-point-scale), which assesses the overall health and pathology of the family.	20-25 weeks ges.	Family affection problems	Epstein, N. B., Baldwin, L. M., Bishop, D. S. (1983). The McMaster family assessment device. Journal of Marital and Family Therapy. 9, (2), 171-180.
				Difficulty in making plans	
				Disapproval of others	
				Difficulty in talking about sadness	
				Avoidance of talking about problems	
				Feelings of being unaccepted	
				Unpleasant and painful feelings	
				Inability to solve problems	
				Decision-making is a problem	
				Distrust of each other	
				Conflicts with each other	
				Family support problems	
	Social Readjustment Rating Scale (SRRS)	See description in the Life Stress Domain.	20-25 weeks ges.	Divorce in the preceding year	-

Supplementary Table 2. Descriptive information of acculturation among national origin groups

National origin group	N	Feeling part of Dutch culture mean (SD)	Language abilities mean (SD)
Dutch	2567	Not applicable	Not applicable
Non-Dutch Western	380	3.9 (1.0)	2.5 (0.7)
Caribbean	426	3.7 (1.0)	2.7 (0.5)
Moroccan/Turkish	532	2.8 (1.2)	2.1 (0.8)
African	197	3.3 (1.1)	2.1 (0.8)
Indonesian	149	4.4 (0.7)	2.9 (0.4)

N= 4251 participants. Higher scores indicate higher levels of acculturation. The variable "Feeling part of Dutch culture" had 5 possible answers ranging from 1, "Not at all", to 5 "Always". The Language abilities variable was graded as 1, "Not Good"; 2, "Reasonable"; or 3, "Very Good".

Supplementary table 3. Non-response analysis

	Study sample (n=4251)		Not in the study sample (non-response at baseline, lost to follow-up and excluded) (n=4725)			Lost to follow-up (n=2168)		
	n	Mean (SD) or %	n	Mean (SD) or %	p- value	n	Mean (SD) or %	p- value
Baseline characteristics								
Maternal Age, years	4251	30.7 (4.9)	4723	28.7 (5.5)	<0.001	2168	29.0 (5.3)	<0.001
Maternal education, %								
Primary	196	4.62	559	13.44	<0.001	209	9.79	<0.001
Secondary	1693	39.96	2072	49.82		1026	48.06	
Higher	2348	55.42	1528	36.74		900	42.15	
Paternal education, %								
Primary	233	5.97	293	10.62	<0.001	147	9.40	<0.001
Secondary	1451	37.19	1157	41.95		618	39.51	
Higher	2218	56.84	1308	47.43		799	51.09	
Child IQ score	4251	102.6 (13.8)	1257	94.67 (16.6)	<0.001	0	-	-
*Child IQ score (including scores<70)	4337	101.9 (14.6)						
Maternal national origin								
Dutch	2567	60.39	1586	37.58	-	992	45.75	-
Indonesian	149	3.51	98	2.32		62	2.86	
Cape Verdian	142	3.34	214	5.07		82	3.78	
Moroccan	214	5.03	369	8.75		143	6.59	
Dutch Antilles	86	2.02	212	5.03		122	5.63	
Surinamese	340	8	431	10.21		215	9.92	
Turkish	318	7.48	460	10.9		196	9.04	
African	55	1.29	125	2.96		45	2.08	
American,western	17	0.4	22	0.52		14	0.65	
European	356	8.38	323	7.66		191	8.81	
Oceanie	7	0.16	2	0.05		2	0.09	
National origins not included:								
American, non western	0	0	120	2.84		32	1.48	
Asian, western	0	0	8	0.19		1	0.05	
Asian, non western	0	0	250	5.92		71	3.27	
Family income, %								
<1200 €	545	14.31	843	28.97	<0.001	434	24.96	<0.001
1200-2000 €	680	17.86	569	19.55		330	18.97	
>2000 €	2583	67.83	1498	51.48		975	56.07	

Supplementary table 3. Non-response analysis (*continued*)

	Study sample (n=4251)		Not in the study sample (non-response at baseline, lost to follow-up and excluded) (n=4725)			Lost to follow-up (n=2168)		
	n	Mean (SD) or %	n	Mean (SD) or %	p- value	n	Mean (SD) or %	p- value
Alcohol frequently in pregnancy, %	4029	9.63 (n=388)	3969	4.79 (n=190)	<0.001	2096	6.77 (n=142)	<0.001
Smoking continued in pregnancy, %	4250	15.32 (n=651)	4291	19.04 (n=817)	<0.001	2168	20.85 (n=452)	<0.001

These analyses were performed in the sample of children whose mothers were prenatally enrolled. n=8976
P-values are from t-tests for continuous variables and Chi-square tests for categorical variables. First, all non-responders (including those at baseline) are compared with the persons in the analyses. Second, the persons who were lost to follow-up are compared with the persons in the analyses. *This sample corresponds to a sensitivity analysis including all child IQ scores

Supplementary table 4. Measurement invariance across groups of maternal national origin (Dutch, non-Dutch Western, non-Western)

	Chi square	Chi-square p-value	CFI	delta CFI	df	Item constrains	Difference p-value
Configural invariance	12,064	0.06	0.995	-	6	none	-
Weak invariance	162,773	<0.001	0.851	0.144	14	All loadings equal between groups.	<0.001
Strong invariance	180,022	<0.001	0.844	0.007	20	All loadings and intercepts equal between groups.	1.00
Strict invariance	594,565	<0.001	0.018	0.826	28	All loadings, intercepts and error variances	<0.001

Performed in the measurement model (latent factor model). All fit measures are scaled (and CFI is robust). Fit indices are the pooled indices (m=40) (n=4251). P-values for the difference in chi-square tests are derived from ANOVAs

Supplementary table 5. Measurement invariance across subgroups of maternal non-Western national origin (Caribbean, Moroccan/Turkish, African, Indonesian).

	Chi-square	Chi-square p-value	CFI	delta CFI	df	Item constrains	Difference p-value
Configural invariance	19,559	0.01	0.979	-	8	none	-
Weak invariance	68,984	<0.001	0.893	0.086	20	All loadings equal between groups.	0.02
Strong invariance	90,244	<0.001	0.875	0.018	29	All loadings and intercepts equal between groups.	1.00
Strict invariance	176,608	<0.001	0.693	0.182	41	All loadings, intercepts and error variances	0.48

Performed in the measurement model (latent factor model). All fit measures are scaled (and CFI is robust). Fit indices are the pooled indices (m=40) (n=1304). P-values for the difference in chi-square tests are derived from ANOVAs

Supplementary table 6. Measurement invariance across Moroccans and Turkish groups

	Chi-square	Chi-square p-value	CFI	delta CFI	df	Item constrains	Difference p-value
Configural invariance	2,967	0.56	1.000	-	4	none	-
Weak invariance	3,434	0.90	1.000	0	8	All loadings equal between groups.	0.99
Strong invariance	7,739	0.74	1.000	0	11	All loadings and intercepts equal between groups.	0.64
Strict invariance	22,181	0.10	0.965	0.035	15	All loadings, intercepts and error variances	0.27

Performed in the measurement model (latent factor model). All fit measures are scaled (and CFI is robust). Fit indices are the pooled indices (m=40) (n=532). P-values for the difference in chi-square tests are derived from ANOVAs

Supplementary table 7. Measurement invariance across the subgroups within the Caribbean group (Dutch Antilles, Surinam)

	Chi-square	Chi-square p-value	CFI	delta CFI	df	Item constrains	Difference p-value
Configural invariance	24,090	<0.001	0.921	-	4	none	-
Weak invariance	22,882	0.004	0.923	-0.002	8	All loadings equal between groups.	0.96
Strong invariance	26,624	0.01	0.918	0.005	11	All loadings and intercepts equal between groups.	0.95
Strict invariance	29,253	0.02	0.916	0.002	15	All loadings, intercepts and error variances	0.95

Performed in the measurement model (latent factor model). All fit measures are scaled (and CFI is robust). P-values for the difference in chi-square tests are derived from ANOVAs (m=40) (n=426).

Supplementary table 8. Measurement invariance across the subgroups within the African group (Cape Verde, Other African)

	Chi-square	Chi-square p-value	CFI	delta CFI	df	Item constrains	Difference p-value
Configural invariance	2,611	0.63	1.000	-	4	none	-
Weak invariance	4,616	0.80	1.000	0	8	All loadings equal between groups.	0.88
Strong invariance	8,920	0.63	1.000	0	11	All loadings and intercepts equal between groups.	0.69
Strict invariance	9,970	0.82	1.000	0	15	All loadings, intercepts and error variances	0.89

Performed in the measurement model (latent factor model). All fit measures are scaled (and CFI is robust). P-values for the difference in chi-square tests are derived from ANOVAs (m=40) (n=197).

Supplementary Table 9. Fit indices of models for the association of Global Maternal Prenatal Stress (latent variable) and Child IQ

	Model Fit Indices											
	Dutch		Non-Dutch Western		Caribbean		Moroccan/Turkish		African		Indonesian	
	AIC	BIC	AIC	BIC	AIC	BIC	AIC	BIC	AIC	BIC	AIC	BIC
Model 1	74908.61	75522.87	13124.43	13537.87	15852.27	16277.74	18625.62	19018.89	7771.58	8115.78	4693.79	4969.53
Model 2	74762.86	75388.82	13111.86	13533.18	15839.92	16273.49	18611.15	19012.98	7774.18	8124.93	-	-
Model 3	74058.54	74754.70	13069.01	13537.57	15722.16	16204.36	18574.16	19027.29	7736.37	8126.47	4544.62	4856.33
Model 4	73978.15	74732.81	13042.60	13550.54	15707.93	16230.65	18561.76	19049.08	7731.00	8153.88	-	-

Fit indices of models examined in table 4. n=4251. The estimates for the Indonesians in model 2 and 4 could not be computed due to negative pooled variance across imputed datasets.

Model 1. Without adjustment

Model 2. Model 1 + Maternal IQ

Model 3. Model 2 + Maternal education + Paternal education + Family income

Model 4. Model 3 + Maternal smoking during pregnancy + Maternal alcohol consumption during pregnancy

Supplementary table 10. Association between the stress domains and child IQ in each national origin group (Analyses in the Dutch and Moroccan/Turkish groups are in Table 5 and 6)

	Child IQ							
	Non-Dutch Western n= 380		Caribbean n= 426		African n= 197		Indonesian n=149	
	b (se)	*P*	b (se)	*P*	b (se)	*P*	b (se)	*P*
Stress variables								
Life stress	0.89 (0.75)	0.23	0.11 (0.53)	0.84	-1.00 (0.84)	0.24	0.45 (1.04)	0.66
Contextual stress	-0.29 (0.87)	0.74	-0.20 (0.59)	0.74	0.68 (1.03)	0.51	-3.07 (1.36)	0.02
Personal Stress	1.17 (0.80)	0.14	-0.20 (0.39)	0.60	-0.42 (0.53)	0.42	1.83 (1.79)	0.31
Interpersonal Stress	-0.25 (0.70)	0.72	-0.32 (0.45)	0.48	0.15 (0.76)	0.84	0.02 (1.29)	0.99

Models were constructed using multiple linear regression (SEM function, MLR estimator). Values are regression coefficients, standard errors and *p* values. The stress variables were standardized. All models converged in at least 30 imputed datasets.

Models were adjusted for Maternal IQ + Maternal education + Paternal education + Family income + Maternal smoking during pregnancy + Maternal alcohol consumption during pregnancy

Supplementary table 11. Association of maternal prenatal stress measures and Child IQ

	Complete sample (n=4251) Model 1		Complete sample (n=4251) Model 2*		Dutch (n=2567)		Non-Dutch Western (n=380)		Caribbean (n=426)		Moroccan/ Turkish (n=532)		African (n=197)		Indonesian (n=149)	
Stress measures	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*
Scales																
BSI, global score	-1.26 (0.57)	0.03	-0.60 (0.59)	0.30	0.74 (1.20)	0.54	1.88 (2.45)	0.44	-1.52 (1.32)	0.25	-2.02 (0.85)	0.02	-0.51 (2.12)	0.81	1.46 (3.91)	0.71
FAD, global score	-1.59 (0.45)	<0.001	-1.22 (0.45)	0.01	-0.70 (0.65)	0.28	-1.37 (1.51)	0.37	-1.77 (1.07)	0.10	-2.95 (1.11)	0.01	-1.12 (1.97)	0.57	3.09 (2.54)	0.22
SRRS, global score	-0.003 (0)	0.39	-0.001 (0)	0.83	0.004 (0.01)	0.44	-0.01 (0.01)	0.68	-0.002 (0.01)	0.82	-0.01 (0.01)	0.21	0.002 (0.01)	0.84	-0.01 (0.01)	0.54
LLDQ, total score	-0.72 (0.74)	0.34	0.07 (0.75)	0.93	1.29 (1.32)	0.33	5.00 (2.41)	0.04	0.31 (1.57)	0.85	-2.08 (1.43)	0.15	-1.76 (2.37)	0.46	-2.16 (3.90)	0.58
Single items																
Personal illness	-0.98 (0.87)	0.26	-0.52 (0.86)	0.54	0.69 (1.71)	0.69	0.71 (2.91)	0.81	-0.63 (1.93)	0.74	-2.12 (1.35)	0.12	-2.59 (3.04)	0.39	-	-
Unplanned pregnancy, yes	-0.79 (0.50)	0.11	-0.41 (0.51)	0.42	0.71 (0.76)	0.35	-1.70 (1.68)	0.31	-0.45 (1.23)	0.71	-2.77 (1.19)	0.02	0.09 (2.06)	0.97	2.56 (2.55)	0.32
Vaginal bleeding in preceding two months, yes	-0.17 (0.90)	0.85	-0.10 (0.89)	0.91	0.48 (1.25)	0.70	4.42 (3.49)	0.21	2.51 (2.57)	0.33	-3.02 (1.75)	0.08	-4.51 (3.76)	0.23	-	-
Chromosomal abnormalities tests, yes	0.83 (0.51)	0.10	0.76 (0.51)	0.13	0.48 (0.61)	0.43	2.65 (1.75)	0.13	3.09 (1.72)	0.07	1.35 (2.17)	0.54	-2.73 (2.97)	0.36	-3.10 (2.11)	0.14
Lack of satisfaction with obstetric care, yes	-2.86 (1.11)	0.01	-2.49 (1.13)	0.03	-3.00 (1.54)	0.05	0.41 (3.54)	0.91	-2.22 (2.68)	0.41	-4.75 (2.68)	0.08	-	-	-	-
Admission to a hospital for >24 hours, yes	-1.08 (1.32)	0.41	-0.83 (1.37)	0.55	-1.87 (1.74)	0.28	-	-	-0.66 (3.08)	0.83	-0.41 (3.09)	0.90	-	-	-	-
Victim of robbery, theft, physical abuse or rape, yes	-1.21 (1.06)	0.25	-1.26 (1.04)	0.23	0.10 (1.44)	0.95	0.06 (3.58)	0.99	0.66 (2.43)	0.79	-5.65 (2.59)	0.03	-8.04 (4.45)	0.07	-	-
Worry about baby's health (POQ), yes	-0.82 (0.52)	0.12	-0.35 (0.53)	0.50	0.13 (0.80)	0.87	1.52 (1.83)	0.41	-1.22 (1.28)	0.34	-1.58 (1.06)	0.14	-0.96 (2.19)	0.66	3.66 (3.21)	0.25
Worry about the pregnancy (POQ), yes	-0.70 (0.55)	0.20	-0.26 (0.56)	0.65	-0.52 (0.84)	0.54	2.79 (1.99)	0.16	0.33 (1.38)	0.81	-2.10 (1.14)	0.07	-2.55 (2.13)	0.23	-	-

Supplementary table 11. Association of maternal prenatal stress measures and Child IQ (*continued*)

	Complete sample (n=4251) Model 1		Complete sample (n=4251) Model 2*		Dutch (n=2567)		Non-Dutch Western (n=380)		Caribbean (n=426)		Moroccan/ Turkish (n=532)		African (n=197)		Indonesian (n=149)	
Lack of housing major appliances, yes	-0.31 (1.02)	0.76	0.26 (1.03)	0.80	-0.65 (2.20)	0.77	0.05 (4.61)	0.99	-0.25 (1.96)	0.90	-1.35 (1.78)	0.45	3.69 (2.87)	0.20	-	-
Housing defects, yes	-0.58 (0.48)	0.22	-0.40 (0.49)	0.41	0.05 (0.66)	0.94	-1.06 (1.68)	0.53	-1.33 (1.29)	0.30	-0.60 (1.18)	0.61	-0.25 (2.28)	0.91	0.12 (2.42)	0.96
Financial difficulties, yes	-1.29 (0.56)	0.02	-0.94 (0.58)	0.11	0.11 (0.89)	0.90	-2.62 (1.86)	0.16	-0.64 (1.50)	0.67	-2.28 (1.10)	0.04	-0.08 (2.16)	0.97	-5.64 (2.91)	0.05
Having a criminal record, yes	-0.84 (2.31)	0.72	-1.03 (2.31)	0.66	-	-	-	-	-	-	-	-	-	-	-	-
Violent interpersonal offenses, yes	-1.65 (1.60)	0.30	-0.91 (1.61)	0.57	-0.74 (3.30)	0.82	-	-	-0.66 (2.16)	0.76	0.77 (4.81)	0.87	-4.01 (3.58)	0.26	-	-
Public order offenses, yes	-3.09 (2.64)	0.24	-2.44 (2.72)	0.37	-	-	-	-	-	-	-	-	-	-	-	-
Early parenthood (age mother < 19 years), yes	0.26 (1.86)	0.89	0.41 (1.88)	0.83	-1.97 (4.16)	0.64	-	-	1.44 (2.99)	0.63	-	-	-	-	-	-
Marital status, single	0.93 (0.70)	0.18	1.37 (0.69)	0.05	2.32 (1.19)	0.05	-3.19 (2.41)	0.19	0.63 (1.35)	0.64	-0.44 (2.52)	0.86	5.47 (2.00)	0.01	3.86 (4.04)	0.34
Large family size, yes	-0.49 (0.58)	0.40	-0.11 (0.58)	0.85	-1.20 (0.95)	0.20	-0.35 (2.12)	0.87	2.42 (1.64)	0.14	-0.13 (1.09)	0.90	0.83 (2.21)	0.71	-4.37 (2.48)	0.08

Models were constructed using multiple linear regression in 40 imputed datasets of the main study sample (n=4251). Values are regression coefficients, standard errors and *p* values. For more information about the stress measures see Supplementary table 1. All models converged in 15 or more imputed datasets. Analyses are only shown for models with more than 10 cases exposed to the stressor.

All models were adjusted for Maternal IQ + SES principal component (Maternal education, Paternal education, Family income) + Maternal smoking during pregnancy + Maternal alcohol consumption during pregnancy. * Model 2 in the complete sample was additionally adjusted for national origin groups (i.e. Dutch, Non-Dutch Western, Non-Western). Adjustment for 6 specific national origin groups was not possible due to convergence problems with imputed datasets.

Abbreviations: BSI = Brief Symptom Inventory. FAD = Family Assessment Device. SRRS = Major stressful life events (Life Events Scale/SRRS). LLDQ = Long Lasting Difficulties (Long Lasting Difficulties Questionnaire). Personal illness = Personal illness (item from the SF-12), moderate/poor health. POQ = Pregnancy Outcome Questionnaire.

Supplementary table 12. Moderation effect of SES on the association of Prenatal Stress and Child IQ

		Interaction SES*stress construct	
National origin groups	**N**	**b (SE)**	***P***
Dutch	2567	-0.13 (0.45)	0.77
Non-Dutch Western	380	-0.98 (1.41)	0.49
Caribbean	426	-2.36 (1.63)	0.15
Moroccan/Turkish	532	-0.40 (1.29)	0.75
African	197	-0.94 (3.14)	0.77
Indonesian	149	-2.05 (2.23)	0.36

These analyses were performed by including an interaction term between the Principal component of SES (based on maternal and paternal education and household income) and the stress latent construct. The stress latent variable and the interaction term were orthogonalized. Estimates were computed based on 40 imputed datasets of our main study sample. n= 4251

The predictors included were: Stress construct + Maternal IQ + SES PC (Maternal education, Paternal education and Family income) + Maternal smoking during pregnancy + Maternal alcohol consumption during pregnancy + Interaction between the stress construct and SES PC.

Supplementary Table 13. Association of Global Maternal Prenatal Stress (latent variable) with Child IQ including child IQ scores below 70

		Child IQ							
National origin group		**Model 1**		**Model 2**		**Model 3**		**Model 4**	
	n	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*	b (SE)	*P*
Dutch	2594	-1.84 (0.37)	<0.001	-1.35 (0.36)	<0.001	-0.41 (0.39)	0.29	-0.29 (0.40)	0.47
Non-Dutch Western	387	-0.73 (1.06)	0.49	-0.39 (1.03)	0.70	0.39 (1.06)	0.72	0.33 (1.05)	0.75
Non-Western									
Caribbean	443	-1.70 (0.72)	0.02	-1.55 (0.71)	0.03	-0.43 (0.78)	0.58	-0.30 (0.79)	0.71
Moroccan/Turkish	550	-1.71 (0.70)	0.01	-1.61 (0.68)	0.02	-1.21 (0.70)	0.08	-1.18 (0.70)	0.09
African	212	0.38 (1.11)	0.73	0.36 (1.12)	0.75	0.95 (1.22)	0.44	0.35 (1.31)	0.79
Indonesian	151	-1.37 (2.45)	0.58	-2.31 (2.48)	0.35	-3.28 (2.17)	0.13	-3.43 (2.23)	0.13

Models were constructed using SEM (Lavaan package). Values are regression coefficients, standard errors and *p* values. The estimates are based on the standardized latent variable (Global Stress). A progressive adjustment was applied to the latent construct and to the structural path as listed below. n=4337.

Model 1. Without adjustment

Model 2. Model 1 + Maternal IQ

Model 3. Model 2 + Maternal education + Paternal education + Family income

Model 4. Model 3 + Maternal smoking during pregnancy + Maternal alcohol consumption during pregnancy

3

Harsh Parenting and Child Brain Morphology: A Population-Based Study

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ABSTRACT

Evidence suggests that maltreatment shapes the child's brain. Little is known, however, about how normal variation in parenting influences the child neurodevelopment. We examined whether harsh parenting is associated with the brain morphology in 2,410 children from a population-based cohort. Mothers *and* fathers independently reported harsh parenting at child age 3 years. Structural and diffusion-weighted brain morphological measures were acquired with MRI scans at age 10 years. We explored whether associations between parenting and brain morphology were explained by co-occurring adversities, and whether there was a joint effect of both parents' harsh parenting. Maternal harsh parenting was associated with smaller total gray (β=-0.05 (95%CI=-0.08; -0.01)), cerebral white matter and amygdala volumes (β=-0.04 (95%CI=-0.07; 0)). These associations were also observed with the combined harsh parenting measure and were robust to the adjustment for multiple confounding factors. Similar associations, although non-significant, were found between paternal parenting and these brain outcomes. Maternal and paternal harsh parenting were not associated with the hippocampus or the white matter microstructural metrics. We found a long-term association between harsh parenting and the global brain and amygdala volumes in preadolescents, suggesting that adverse rearing environments common in the general population are related to child brain morphology.

INTRODUCTION

A growing body of research in clinical samples suggests that the exposure to early adverse caregiving is associated with child neurodevelopment. In particular, an effect of early-life maltreatment and traumatic events on the limbic morphology has been postulated. The amygdala and hippocampus are brain regions of interest in the context of adverse caregiving for several reasons. First, both structures undergo a period of rapid development in early childhood (Uematsu et al., 2012), and thus adverse caregiving environments coinciding with this developmental timing could influence the developmental trajectory (Tottenham & Sheridan, 2010). Second, as described by Tottenham and Sheridan (2010), the amygdala and hippocampus have a high density of cortisol receptors and therefore may be affected by variation in levels of this stress hormone. In fact, cortisol has been shown to influence the neurogenesis (Odaka et al., 2017), thus representing a pathway through which stressful environments could shape brain morphology. Finally, in addition to the biological relatedness between the limbic structures and the stress response, a functional relation exists. The amygdala plays a key role in the response to emotional stimuli (Bonnet et al., 2015) and fear conditioning (Milad & Quirk, 2012), whereas the hippocampus is involved in the memory encoding (Schiller et al., 2015) and the termination of the stress response (McEwen & Akil, 2020). Further, differential patterns of amygdala and hippocampal activation have been described among children exposed to threat (McLaughlin et al., 2019).

Based on animal and human studies, several potential mechanisms that link early life adversity with child brain development have been proposed. First, childhood trauma has been associated with the development of inflammation, and there is evidence supporting the influence of the immune system on brain morphology through an effect on the development of axons, synapses and the production of myelin (Danese & Lewis, 2017). Second, traumatic events have been related to oxidative stress in the central nervous system. Oxidative stress, defined as the excess of reactive oxygen species compared to the neutralizing antioxidant response, may lead to alterations in brain morphology, cause neuroinflammation and even generate neuronal death (Schiavone et al., 2013). Third, a disruption of the stress-response systems, including the hypothalamic-pituitary-adrenal (HPA) axis, has been suggested to explain the associations between adversities and brain differences (Wesarg et al., 2020). This latter mechanism likely occurs in parallel and in close relation with the previous two, and may exert an effect in the brain morphology through the secretion of catecholamines and glucocorticoids (Wesarg et al., 2020). As posited by the Allostatic Load Model, these different mechanisms may be activated in normal responses to stressful events, offering an adaptive and protective response (McEwen, 1998). However, when the exposure to stress is sustained, these mechanisms may be overstimulated and lead to a pathophysiological response (McEwen, 2001;

McEwen & Akil, 2020). This maladaptive effect, termed "allostatic load" (McEwen & Akil, 2020), may generate neurotoxicity and volumetric reduction of multiple brain regions through processes such as neuronal damage, and dendritic remodeling (Kim et al., 2015; McEwen, 2001).

Overall, most research suggests that child exposure to adverse caregiving conditions is related to smaller volumes of the amygdala and hippocampus (see for a review: McLaughlin et al. (2019)). Also, associations between child maltreatment and smaller total brain, grey and white matter volumes have been described (McLaughlin et al., 2016; Teicher & Samson, 2016).

In comparison to the literature on extreme adverse caregiving, substantially less is known about the normative variation of harsh parenting. Whittle et al. (2009) described a cross-sectional relation between punishing maternal behaviors and *larger* amygdala and regional cortical volumes in 12-years-old children (N=113). Maternal parenting was also assessed by Blankenship et al. (2019), who found that children exposed to negative parenting in early childhood had smaller volumes of the hippocampus tail at ages 5-10 years (N=63). Few studies examined the association between adverse caregiving and white matter microstructure. One diffusion tensor imaging (DTI) study of 32 adults described a relation between parental verbal abuse and reduced fractional anisotropy (FA) of several white matter tracts, including the cingulum bundle (Choi et al., 2009). Additionally, childhood abuse was shown to be associated with reduced FA of the inferior fronto-occipital fasciculus in a sample of 63 youth (Lim et al., 2019).

Several aspects limit the comparability across studies. Whereas some assessed harsh parenting in early childhood, others measured it in pre-adolescence, and studies also differed in the brain outcomes examined. Moreover, most studies had a small-to-moderate sample size, and some oversampled participants with high risk for mental disorders (Blankenship et al., 2019; Whittle et al., 2009), limiting the generalizability of results.

Sex-specific associations have been described in relation to the brain vulnerability to environmental factors in early life. In particular, some maltreatment studies have reported greater brain morphological differences in males than in females, suggesting that some structures may be more susceptible to early-life stress in males (see for a review: Teicher and Samson (2016)). Thus, the sex-specificity of the association between parenting and child brain morphology should be considered.

A gap in the existing literature is the lack of research on paternal parenting, although evidence supports a role of fathers in offspring development. Both maternal and paternal sensitivity were associated with offspring brain differences in the present cohort (Kok et al., 2015). Additionally, an interaction effect has been described for maternal and paternal harsh parenting in relation to the offspring outcomes (Meunier et al., 2012; Wang et al., 2019). For example, children exposed to negative parenting by both parents have been shown to have the highest levels of emotional problems (Meunier et al.,

2012). The primary aim of this study was to examine whether maternal and paternal harsh parenting behavior were associated with child brain morphology. In additional analyses, we addressed the relation between the combined maternal and paternal harsh parenting exposure and the child brain outcomes, and the interaction between both parental harsh parenting measures.

In 2,410 10-year-old children from the general population, we examined the relation between early-life harsh parenting and child brain morphology. Given that harsh parenting may be considered a chronic exposure to adverse caregiving conditions, we hypothesized parental harsh parenting would be associated with smaller amygdala and hippocampal volumes. We also examined the cortical thickness and the global brain volumes. Building on existing evidence, we expected to find an association between harsh parenting and smaller global brain volume measures. Further analyses with white matter microstructural metrics were performed with an exploratory approach given the scarcity of previous evidence. Also, we tested whether child sex modified the relation of harsh parenting with brain morphology.

METHOD

Participants

This study is part of the Generation R Study, a population-based cohort that follows the development of children in Rotterdam, the Netherlands (Kooijman et al., 2016). The design of the cohort has been previously described in detail (Jaddoe et al., 2006). Briefly, pregnant women residing in the study area with a delivery date from April 2002 to January 2006 were eligible. They received information about the study from midwives and obstetricians and were contacted by study researchers for additional information (Jaddoe et al., 2006). In total, 9,778 mothers were enrolled (response rate of 61% at birth).

The cohort study includes families with various national origins (Dutch as the majority group). Mothers with higher socioeconomic status were more likely to participate. The aim of this ongoing cohort study is to identify environmental and genetic factors that influence children's growth, development and health. Thus, data on multiple child and parent characteristics, including biological and psychological factors, was collected. The study was approved by the Medical Ethical Committee of the Erasmus Medical Center and all participating parents gave informed consent.

Of the 4,974 children with information on maternal and/or paternal harsh parenting at age 3 years, 2,801 had neuroimaging data at age 10 years. For the analyses with structural MRI (magnetic resonance imaging), we excluded 521 children with poor image quality and 9 with major incidental findings in the MRI scans. For the analyses with DTI metrics, we excluded 556 children with non-usable DTI data and 8 children with major

incidental findings. We also randomly excluded siblings to avoid bias due to paired data (N=147). In total, 2,410 children were included in analyses (2,141 with structural MRI data and 2,108 with DTI data; Supplementary Figure 1).

Measures

Harsh Parenting

Information on harsh parenting practices was collected when children were 3 years old using questionnaires based on the Parent-Child Conflict Tactics Scale (CTSPC) (Straus et al., 1998). Items on harsh punishment (e.g. spanking) originally included in the CTSPC were removed, as these practices may be considered illegal in the Netherlands and we had no mandate to follow-up on such practices. Additionally, one item that was not age-appropriate ("said you would kick child out of the house") was removed. Mothers and fathers independently reported the use of various harsh parenting practices in the preceding 2 weeks (see Supplemental Material), using a 6-point frequency scale (from *Never* to *More than four times*). In a previous study from this cohort, Jansen et al. (2012) described the selection of items for the harsh parenting measure. Briefly, an exploratory factor analysis on the ten items included (using a 3-point frequency scale) showed a two-factor structure, and the six items of the first factor, with factor loadings >0.50, matched the construct and definition of harsh parenting (Jansen et al., 2012). We computed maternal and paternal harsh parenting scores by summing the scores on the six harsh parenting items (range=0-30). The internal consistency of both maternal and paternal harsh parenting was low (Cronbach's alpha of 0.63 in the total sample, and in the study sample 0.60 for maternal harsh parenting, and of 0.58 for paternal harsh parenting), likely reflecting the small number of items in these scales. Importantly, the six items of the harsh parenting measure showed good model fit in both mothers (comparative fit index (CFI)= 0.970, Tucker-Lewis index (TLI)= 0.966, root mean square error of approximation (RMSEA)= 0.044) and fathers (CFI= 0.972, TLI= 0.965, RMSEA= 0.040) (Jansen et al., 2012). Several determinants and correlates of harsh parenting (e.g. socioeconomic status, family dysfunction, child behavioral problems) have been identified in the current cohort (Jansen et al., 2012; Mackenbach et al., 2014) supporting the validity of our harsh parenting measure.

Brain Imaging

Acquisition:

Magnetic resonance images were acquired at age 9-11 years using a 3-Tesla General Electric scanner (MR750w, Milwaukee, WI, US), with signal reception through an 8-channel head coil (White et al., 2018). T_1-weigthed images were collected with an Inversion

Recovery Fast Spoiled Gradient Recalled sequence (TR=8.77ms, TE=3.4ms, TI=600ms, Flip angle=10°, Field of View (FOV)=220x220mm, Acquisition matrix=220x220, Slice thickness=1mm, Number of slices=230, ARC acceleration factor=2). The diffusion-weighted images were collected using an echo planar sequence with 3 b=0s/mm^2 volumes and 35 diffusion-weighted images (TR=12.500ms, TE=72.8ms, FOV=240x240mm, Acquisition matrix=120x120, slice thickness=2mm, number of slices=65, ARC acceleration factor=2 and b=900s/mm^2).

Image processing:

Images were processed with the FreeSurfer version 6.0 image suite (http://surfer.nmr.mgh.harvard.edu/), as previously described (Muetzel et al., 2019). In brief, we performed removal of non-brain tissue, voxel intensity normalization, volumetric segmentation and cortical reconstruction. Cortical thickness was estimated for each vertex as the distance between the grey/white matter boundary and the grey matter/cerebrospinal fluid boundary. Thickness maps were smoothed with a 10mm full-width half-maximum Gaussian kernel. Image quality of the FreeSurfer reconstructions was assessed as described previously (Muetzel et al., 2019). Further details of the image quality control are described in the Supplement Material. We included the total grey and cerebral white matter volumes, amygdala and hippocampal volumes (averaged over both hemispheres) and cortical thickness vertex-wise data in analyses. The hemisphere-specific amygdala and hippocampus were examined in sensitivity analyses.

The DTI data was processed using the FMRIB Software library (FSL)(Jenkinson et al., 2012) and the Camino toolkit (Cook et al., 2006). We removed non-brain tissue and corrected the images for eddy-current artifacts and translations/rotations caused by head motion. The diffusion tensor was fitted and fully-automated probabilistic tractography was run, generating connectivity distributions for multiple white matter tracts. Average fractional anisotropy (FA) and mean diffusivity (MD) values were computed for each tract, weighted by the connectivity distributions, and global FA and MD metrics were derived from the metrics of multiple large fiber bundles with confirmatory factor analysis (Muetzel et al., 2018). We used the global FA and MD factor scores. Detailed quality control of the brain images was performed and data rated as inadequate were excluded from analyses (see Supplemental Material).

Covariates

Potential confounders were selected based on previous studies (Kok et al., 2015; Whittle et al., 2016). Marital status, maternal national origin, prenatal smoking and alcohol consumption were self-reported with questionnaires during pregnancy. Information on child birth weight was collected from hospital registries and midwives. Maternal and paternal education were assessed prenatally and at age 3 years. Family income and

parental depressive symptoms were self-reported at age 3 years. Maternal and paternal depressive symptoms were assessed with the depression subscale of the Brief Symptom Inventory (BSI)(Derogatis, 1993), a validated questionnaire that assesses psychiatric symptoms. The total intracranial volume was extracted from the structural imaging data (Additional information in the Supplemental Material).

Maternal alcohol drinking problems and marital problems were included in sensitivity analyses. Information on maternal alcohol consumption was collected by postal questionnaires when children were 5 years old. If mothers reported drinking any alcohol over the past three months, several follow-up questions were asked to examine the drinking pattern. We distinguished two problematic maternal alcohol drinking patterns: "regular drinking problems", defined as drinking more than one glass of alcohol a day on average (vs no alcohol consumption or consumption of one or fewer alcohol glasses per day), and "binge drinking", defined as drinking more than 6 glasses in one day more than once a month (vs drinking more than 6 glasses in one day less than once a month, or no consumption of more than 6 glasses in one day). Regarding marital problems, the primary caregiver (in most cases the mother) reported at child age 3 years whether problems in the couple relationship had occurred (yes/no) in the preceding two years.

Statistical Analyses

We examined whether the maternal and paternal harsh parenting scores were related to the regions of interest (ROIs, i.e. total grey and cerebral white matter volume, mean amygdala and hippocampal volumes; and global FA and MD) with linear regression. We controlled for confounders in two models. First, analyses were adjusted for total intracranial volume (in models with the amygdala and hippocampus), child age at the MRI scan, child sex, and maternal national origin. In a second model, we additionally controlled for birth weight, prenatal smoking and alcohol consumption, family income, maternal education, marital status and maternal depressive symptoms. Analyses with the paternal harsh parenting included paternal education and paternal depressive symptoms instead of the respective maternal covariates. Similar models were fitted to examine the association of parenting with cortical thickness at each cortical vertex (QdecR package, version 0.8.4, https://github.com/slamballais/QDECR)(Muetzel et al., 2019). We tested the interaction between child sex and (maternal and paternal) harsh parenting for the ROIs and followed-up significant results with sex-stratified analyses.

The eight analyses with the structural ROIs (four tests for each parent's harsh parenting) and the eight analyses of the interaction between child sex and harsh parenting (four tests for each parent's harsh parenting) were adjusted for multiple testing with the false discovery rate approach (FDR)(Benjamini & Hochberg, 1995). The vertex-wise analyses were adjusted for multiple testing using Gaussian Monte Carlo Simulations (Hagler

et al., 2006) with a cluster-forming threshold (CFT) of p=0.001 (Greve & Fischl, 2018) and a cluster-wise p-value (CWP) of p<0.025 (Bonferroni-corrected for both hemispheres).

We performed several additional analyses, fully-adjusted for covariates (i.e. total intracranial volume (in amygdala and hippocampus analyses), child sex and age at the MRI scan, maternal national origin, birth weight, prenatal smoking and alcohol consumption, family income, maternal education, marital status and maternal depressive symptoms. In analyses with paternal harsh parenting, paternal education and depressive symptoms were included instead of the respective maternal covariates). First, as the amygdala and hippocampal volumes follow hemisphere-specific developmental trajectories (Uematsu et al., 2012), we examined left- and right-hemisphere measures in independent analyses. Second, we explored whether there was an interaction between maternal and paternal harsh parenting for the brain ROIs. Third, we explored the relation between the combined parental harsh parenting measure and child brain morphology. To this aim, we performed a principal component analysis (PCA), based on the original items of the harsh parenting maternal and paternal measures (6 items per parent; missing values imputed with the median). Given that the purpose of this analysis was to combine maternal and paternal harsh parenting metrics in one measure, only the first component was extracted. The association between this combined parental harsh parenting measure and the child brain outcomes was examined with linear regression, fully adjusted for confounders (additionally, both maternal and paternal education and depression were included as covariates). Fourth, we examined whether our findings were explained by two other stressful experiences: maternal alcohol drinking problems, and marital problems. Parental alcohol abuse has been suggested to influence child psychological development (Raitasalo et al., 2019), and the likelihood of child maltreatment is higher in families where parents abuse alcohol (Dube et al., 2001). Similarly, family dysfunction has been associated with more parental harsh discipline (Jansen et al., 2012) and with offspring brain morphology (Xerxa, Delaney, et al., 2020). We further explored associations observed in the main analyses, by adjusting, first, for maternal regular drinking problems and binge drinking; and second, for marital problems.

All analyses were run with the statistical software R (version 3.6.1)(R Core Team, 2020). Estimates were standardized for ease of interpretation. Missing values in covariates (maximum missingness: Paternal depressive symptoms: 19.4%) were imputed with the Multivariate Imputations by Chained Equations (mice) package (version 3.6.0)(van Buuren & Groothuis-Oudshoorn, 2011) generating 20 imputed datasets. One participant with an outlying global MD score (>4 standard deviations below the mean) was excluded from the DTI analyses.

Non-response Analysis

Children in the analyses (N=2,410) did not differ from children lost-to-follow-up (with harsh parenting data but no neuroimaging data, N=2,173) in sex and maternal marital status. Children included in analyses were exposed to less harsh parenting by mothers and fathers than children with no imaging data (e.g. mean maternal harsh parenting: 2.88 vs 3.11, p=0.02). Of the children in our study, 35% had highly-educated mothers whereas this was of 33% in those lost to follow-up (chi-square p=0.02). Likewise, 66% of the children in our study sample had mothers with Dutch national origin, whereas this was of 63% in the lost-to-follow-up group (p=0.02).

RESULTS

Among the 2,410 children in analyses, 51% were girls. The correlation between maternal and paternal harsh parenting was moderate (Pearson's r=0.36, p<0.001, N=1,905). The median (unstandardized) maternal harsh parenting score was the same for boys and girls (median=2.0, IQR=1.0, 4.0), whereas the median paternal harsh parenting score was 2.0 (IQR=1.0, 3.0) for boys and 1.0 (IQR=0, 3.0) for girls. Most mothers were married or living with a partner (91%) and 35% of mothers and 37% of fathers were highly educated (Table 1).

The exposure to maternal harsh parenting was associated with smaller total gray matter (β= -0.07 (95% confidence interval (95%CI)=-0.10; -0.03)) and cerebral white matter volumes (β= -0.06 (95%CI=-0.09; -0.02)) after adjusting for child age at the MRI scan, child sex, and maternal national origin. These associations remained after additionally accounting for birth weight, prenatal smoking and alcohol consumption, family income, maternal education, marital status and maternal depressive symptoms (total gray matter volume: β= -0.05 (95%CI=-0.08; -0.01)). Maternal harsh parenting was also related to smaller amygdala volumes (β= -0.04 (95%CI=-0.07; 0)), but not to hippocampal volumes. No association was found between maternal harsh parenting and global white matter microstructural metrics (Table 2).

Paternal harsh parenting had similar direction of effects as maternal harsh parenting for the associations with global brain volumes (e.g. cerebral white matter volume (β= -0.03 (95%CI=-0.07; 0.01)) and amygdala volume (β= -0.03 (95%CI=-0.07; 0.01)). However, these associations were not statistically significant. Similarly, no association was found between paternal harsh parenting and hippocampal volume or white matter microstructural metrics (Table 2).

Table 1
Baseline characteristics

	mean (SD) or %*
Child characteristics	
Sex, % girls	50.7
Age at the MRI scan, years	10.1 (0.5)
Age at maternal harsh parenting measure, years (N=2358)	3.0 (0.1)
Parental characteristics	
Maternal characteristics	
Harsh parenting, maternal score, median (Q1, Q3) (N=2358)	2.0 (1.0, 4.0)
Education, %	
Low	37.1
Medium	28.3
High	34.6
Maternal national origin, %	
Dutch	65.7
Non-Western	21.7
Non-Dutch Western	12.6
Marital status, % married or living together	91.4
Prenatal smoking, % never during pregnancy	79.8
Prenatal alcohol use, % never during pregnancy	34.7
Depression symptoms, BSI depression score, median (Q1, Q3)	0 (0, 0.17)
Paternal characteristics	
Harsh parenting, paternal score, median (Q1, Q3) (N=1957)	1.0 (0.0, 3.0)
Education, %	
Low	39.4
Medium	24.0
High	36.6
Depression symptoms, BSI depression score, median (Q1, Q3)	0 (0, 0.01)

Note. Sample with available data for maternal and/or paternal harsh parenting and brain T1 and/or DTI MRI (N=2410) *Otherwise indicated.

After adjustment for multiple testing in the analyses with maternal and paternal harsh parenting and the brain structural regions of interest (eight tests), only the association between maternal harsh parenting and total gray matter volume survived (p-adjusted=0.05). The associations of maternal parenting with cerebral white matter (p-adjusted=0.09) and amygdala volumes (p-adjusted=0.09) did not survive. No associations were found between maternal or paternal harsh parenting and vertex-wise cortical thickness.

Table 2

Harsh parenting and the child brain outcomes

	Maternal harsh parenting					Paternal harsh parenting				
		Model 1		Model 2			Model 1		Model 2	
	N	ß (95%CI)	P	ß (95%CI)	P	N	ß (95%CI)	P	ß (95%CI)	P
Brain Outcomes										
Global brain measures	2090					1754				
Total gray matter volume		-0.07 (-0.10; -0.03)	<0.001	-0.05 (-0.08; -0.01)	0.006		-0.03 (-0.07; 0.01)	0.152	-0.02 (-0.06; 0.02)	0.298
Cerebral white matter volume		-0.06 (-0.09; -0.02)	0.003	-0.04 (-0.08; 0)	0.035		-0.03 (-0.07; 0.01)	0.100	-0.03 (-0.07; 0.01)	0.177
Subcortical structures										
Amygdala volume		-0.04 (-0.07; 0)	0.043	-0.04 (-0.07; 0)	0.028		-0.02 (-0.06; 0.01)	0.226	-0.03 (-0.07; 0.01)	0.124
Hippocampus volume		-0.02 (-0.06; 0.01)	0.200	-0.02 (-0.05; 0.02)	0.286		0 (-0.04; 0.04)	0.924	0 (-0.04; 0.03)	0.835
Global DTI measures	2064					1720				
Global FA		-0.03 (-0.07; 0.01)	0.198	-0.02 (-0.07; 0.02)	0.275		0 (-0.05; 0.04)	0.898	-0.01 (-0.05; 0.04)	0.763
Global MD		-0.01 (-0.05; 0.04)	0.758	-0.01 (-0.05; 0.04)	0.743		0.01 (-0.04; 0.05)	0.710	0.01 (-0.03; 0.06)	0.585

Note. Associations between parental harsh parenting at age 3 years and child brain outcomes at age 10 years. Amygdala and hippocampal volumes averaged over both hemispheres. Model 1 is adjusted for: total ICV (total intracranial volume), child age at brain MRI scan, child sex and maternal national origin. Model 2 is additionally adjusted for birth weight, prenatal smoking and alcohol consumption, family income, maternal education, marital status and maternal depressive symptoms. In paternal harsh parenting analyses, paternal education and depressive symptoms are included instead of the maternal covariates. Global brain measures are not adjusted for total ICV. Estimates are standardized.

We examined whether the relation between harsh parenting and the ROIs differed by child sex. However, no interaction effect was found between harsh parenting and the child sex for any of the brain outcomes examined (data not shown).

Next, we explored whether the associations between harsh parenting and amygdala and hippocampal volumes were hemisphere-specific. Maternal harsh parenting was consistently related to the left and right amygdala volumes (left: β= -0.04 (95%CI=-0.08; 0); right: β= -0.03 (95%CI=-0.07; 0)), although the analyses with the right amygdala were not statistically significant. We found similar estimates for the relation of paternal harsh parenting and the amygdala volumes (e.g. left: β= -0.03 (95%CI=-0.07; 0.01)), which did not reach significance. There was no association between each parent's harsh parenting and the hippocampal volumes (Supplementary Table 1).

To further explore the role of maternal and paternal harsh parenting in the relation with the child brain morphology, we performed two sensitivity analyses. First, we examined the interaction between maternal and paternal harsh parenting. We found no evidence for an interaction effect in relation to any of the brain outcomes examined (Table 3). Second, we modelled the joint effect of maternal and paternal harsh parenting, by performing a PCA based on the 12 items of the maternal and paternal harsh parenting reports. We extracted the first component, explaining 24% of the total variance, with an eigen value of 2.87. Factor loadings ranged from 0.31 to 0.57 for all items. We then examined the association between the harsh parenting factor score and the brain outcomes. Parental harsh parenting was related to smaller total gray matter volume (β= -0.04 (95%CI=-0.07; 0.00)) and amygdala volume (β= -0.04 (95%CI=-0.07; -0.01)) in analysis adjusted for all covariates. A suggestive, although non-significant association was observed between the parental harsh parenting measure and cerebral white matter volume (Supplementary Table 2). Considering the moderate correlation between maternal and paternal harsh parenting and the relatively low percentage of explained variance by the extracted principal component, we recommend caution in the interpretation of these results.

Finally, we explored whether our findings were explained by two potentially co-occurring stressors. We followed-up the associations of maternal harsh parenting with total gray matter, cerebral white matter and amygdala volumes, by adjusting for maternal alcohol drinking problems and for the presence of marital problems. However, neither of these factors even partly explained the associations between maternal harsh parenting and child brain morphology (Supplementary Table 3).

Table 3

Interaction between maternal and paternal harsh parenting in relation to child brain morphology

		Interaction Model					
		Maternal Harsh Parenting		Paternal Harsh Parenting		Interaction maternal*paternal harsh parenting	
	N	ß (95%CI)	P	ß (95%CI)	P	ß (95%CI)	P
Brain Outcomes							
Global brain measures							
Total gray matter volume	1703	-0.04 (-0.08; 0.01)	0.104	-0.02 (-0.06; 0.02)	0.329	0 (-0.03; 0.03)	0.897
Cerebral white matter volume	1703	-0.03 (-0.08; 0.01)	0.188	-0.04 (-0.08; 0.01)	0.093	0.01 (-0.02; 0.04)	0.406
Subcortical structures							
Amygdala volume, average	1703	-0.03 (-0.07; 0.02)	0.234	-0.04 (-0.08; 0)	0.071	0 (-0.03; 0.03)	0.845
Hippocampus volume, average	1703	-0.03 (-0.07; 0.01)	0.182	-0.01 (-0.06; 0.03)	0.522	0.02 (-0.01; 0.05)	0.126
Global DTI measures							
Global FA	1677	-0.04 (-0.1; 0.01)	0.129	0 (-0.05; 0.06)	0.898	0.01 (-0.02; 0.04)	0.500
Global MD	1677	0.01 (-0.04; 0.07)	0.649	0 (-0.05; 0.05)	0.978	-0.01 (-0.04; 0.03)	0.730

Note. Predictors included: maternal and paternal harsh parenting, total ICV (total intracranial volume), child age at MRI scan, child sex, maternal national origin, birth weight, prenatal smoking and alcohol consumption, family income, maternal education, paternal education, marital status, maternal depressive symptoms, paternal depressive symptoms and **an interaction term between maternal and paternal harsh parenting**. Global brain measures are not adjusted for total ICV. Estimates are standardized.

DISCUSSION

In this population-based study, early-life maternal harsh parenting was associated with smaller total gray matter volumes in 10-year-old children. These results were robust to the adjustment for multiple confounding factors, and were not explained by the presence of other child stressful experiences. Similar associations were observed for the cerebral white matter and the amygdala volumes. However, these findings did not survive after adjustment for multiple testing. The associations between paternal harsh parenting and child brain morphology showed the same direction and largely similar effect sizes as maternal harsh parenting, but did not reach significance. Further, analyses with a joint parental harsh parenting measure showed results consistent with those of the separate maternal and paternal analyses: parental harsh parenting was associated with smaller global brain and amygdala volumes. Differences in the hippocampal volumes were not related to past harsh parenting exposure. Also, parental harsh parenting was not associated with regional cortical thickness or white matter microstructural metrics.

Multiple studies have examined the brain morphology of children exposed to severe early-life adverse caregiving conditions and have consistently found that children who experienced adversity, such as maltreatment, have smaller global brain volumes than controls, with wide-spread differences observed in grey and white matter (Bick & Nelson, 2016; De Brito et al., 2013). In this large population-based cohort, we examined whether harsh parenting, which can be conceptualized along a continuum of parenting with maltreatment at the extreme end (Gershoff, 2002; Kim et al., 2010), was related to the child brain morphology. Interestingly, our results are in line with the existing maltreatment literature: harsh discipline was associated with smaller global brain volumes. Contrary to what we expected, harsh parenting was not related to child cortical thickness. Thinner cortices in specific regions, such as the prefrontal cortex, have been described by some studies of children exposed to severe caregiving adversity (McLaughlin et al., 2019). Yet, even though we observed global brain volumetric differences in relation to harsh parenting, no specific association with cortical thickness was found. Given the population-based design of our study, cortical thickness differences could be too subtle to be detected with our current sample size. It is also possible that the observed global differences are accounted for by differences in other components of grey matter rather than cortical thickness, such as the cortical surface area or the local gyrification (Kelly et al., 2013). Our findings add to the evidence linking harsh parenting with subsequent offspring behavioral problems (Jackson & Choi, 2018; Mackenbach et al., 2014), demonstrating a difference in child grey matter volumes. Research has shown that sustained exposure to stress can lead to allostatic load, and trigger pathophysiological reactions at the endocrine and molecular levels, among others (McEwen & Akil, 2020). Thus, the smaller grey matter volume could be related to neurotoxicity and dendritic remodeling,

caused by a maladaptive stress response. Further studies are needed to better understand how brain morphology correlates at the local neuroanatomical level and how this corresponds to the association of parental harsh discipline with subsequent poor child outcomes.

The literature shows mixed results regarding the relation of early-life adverse caregiving with amygdala volume. Some research results suggest that the amygdala may be smaller in children exposed to severe adverse caregiving (McLaughlin et al., 2016), but larger amygdala volumes have also been described (Whittle et al., 2009). We report that harsh parenting was associated with smaller amygdala volumes, and this finding was consistently observed in the left and right hemisphere. Overall, it is difficult to compare findings across studies given the differences in age and measurements. For example, Whittle et al. (2009) examined the relation of mothers' punishing responses in reaction to adolescents' positive affective behavior with adolescents' brain morphology. Further, this parental behavior pattern was most probably related to the adolescents' neural circuitry of reward. In contrast, our study focused on parenting of 3-year-old children and examined the daily-life use of harsh discipline strategies, which are often seen as related to child maltreatment (Stith et al., 2009). Additionally, the age at the brain MRI assessment could influence the results, considering that the amygdala has a non-linear developmental trajectory plateauing in preadolescence (Uematsu et al., 2012). Importantly, our finding of a relation between harsh parenting and a smaller amygdala volume in children expands the existing evidence regarding adverse caregiving environments in the general population. Further, the experience of maltreatment has also been related to the functional connectivity between the amygdala and the prefrontal cortex, suggesting that early-life adversity could be related not only to the amygdala morphology, but also to its functional reactivity (Peverill et al., 2019).

It is well known that adverse experiences tend to co-occur (Felitti et al., 1998). Factors such as low SES (Roubinov & Boyce, 2017), alcohol drinking problems (Dube et al., 2001) and marital problems (Jansen et al., 2012) predict the use of harsh discipline strategies, and are related to child brain and psychological development (McDermott et al., 2019; Raitasalo et al., 2019; Xerxa, Rescorla, et al., 2020). Sensitivity analyses showed that our findings were not explained by these potentially co-occurring stressful factors. Rather, we hypothesize that harsh parenting represents a chronic stressor, that in the long term may alter the brain's developmental trajectory through a cascade of disruptions in the stress response system and in the physiological responses to external events (Evans et al., 2013). The prolonged exposure to stress has been suggested to alter neuronal morphology, the normative trajectory of neuronal proliferation, and synaptic plasticity (Kim et al., 2015).

Interestingly, mothers' and fathers' parenting were similarly related to the child brain outcomes, although the association of the father's parenting was attenuated. Differen-

tial parenting practices have been described for mothers and fathers (McKinney & Renk, 2008), yet, little is known regarding the relation of *paternal* parenting with the child brain morphology. Our study gives only a preliminary answer to this question. Given the smaller sample size of children with paternal parenting reports than maternal reports and the smaller effect sizes of the associations with the brain outcomes, it is possible that larger sample sizes are needed to observe a slightly subtler effect. The analyses with the joint parental harsh parenting measure supported a joint effect of maternal and paternal parenting, suggesting that the exposure to more harsh parenting from *both* parents is related to similar brain differences as those observed in the separate maternal and paternal analyses. Additionally, some researchers suggest that the harsh discipline of mothers and fathers could interact in relation to the offspring outcomes (Wang et al., 2019). However, we found no evidence for a maternal and paternal harsh parenting interaction effect in relation to the brain regions of interest. Also, some studies have described that boys may be more susceptible to poor parenting than girls (Spruijt et al., 2019), but we observed no interaction of maternal and paternal harsh parenting with child sex.

In this study, we found no association between parental harsh parenting and the hippocampal volumes. Although the literature is not very consistent, some studies have reported smaller hippocampal volumes in children exposed to early adversity (Bick & Nelson, 2016; McLaughlin et al., 2019). One study found that early-life negative parenting predicted smaller hippocampal tail volumes via cortisol reactivity, suggesting that stress reactivity may underlie the relation between parenting and offspring neurodevelopment (Blankenship et al., 2019). Moreover, extreme caregiving adversity has been related to deficits in memory (Bick & Nelson, 2016) and other hippocampal-related cognitive tasks (Edmiston & Blackford, 2013). Given that the hippocampus and amygdala have a period of rapid growth and development during early childhood (Uematsu et al., 2012), this may represent a period of critical vulnerability of these limbic structures to environmental effects. Thus, the lack of association between harsh parenting and the hippocampal volume in our study could simply reflect the fact that larger study samples of children from the general population are needed to detect small but possibly relevant hippocampal volumetric differences, and that these may be more apparent in children exposed to severe adverse caregiving conditions.

Similarly, we found no association between harsh parenting and the global white matter microstructural metrics in our exploratory analyses. While childhood abuse studies reported white matter microstructural differences in adults (Lim et al., 2019), further studies in children and in the general population are needed to understand the relation between caregiving adversity and child white matter microstructure.

Our findings must be interpreted considering some limitations. First, causality cannot be inferred. Future studies should include repeated parenting and neuroimaging

measures, to examine the direction of effect. Second, harsh parenting measures were based on self-reports, which could be biased by social desirability. However, observational parenting assessments also have a tendency towards socially desirable behaviors, and other data collection methods, like child reports, are especially challenging when assessing harsh parenting in early childhood (Bennett et al., 2006). Third, children lost-to-follow-up less often had mothers with a Dutch national origin and high education than children included in our study. Moreover, the relatively high educational level of families in our cohort study and the low poverty rate in the Netherlands (2019) may have limited the variation in our harsh parenting measure. Fourth, paternal harsh parenting data was less often available than maternal parenting. Although our sample is large compared to previous studies, and that there was an overall consistency of effect between both parents, larger population-based samples could be needed to capture subtle effects. Fifth, alcohol consumption was collected by postal questionnaires, which could have led to an underestimation of the amount of consumed alcohol.

Our findings in this population-based study suggest that early-life harsh parenting is related to smaller global brain and amygdala volumes in preadolescence. These results have public health relevance as these offer an extension of the evidence of child maltreatment studies, suggesting that adverse rearing environments common in the general population are related to child brain morphology.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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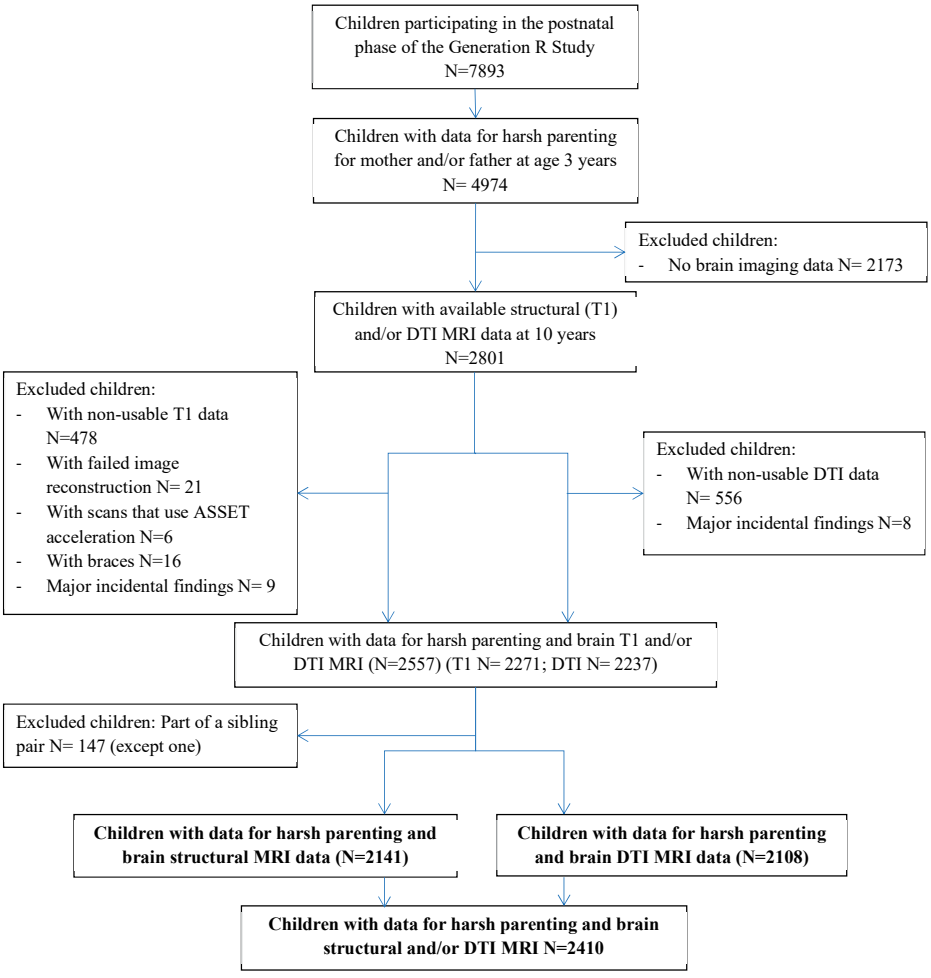
SUPPLEMENTAL MATERIAL



Supplementary Figure 1. *Sample selection*

Supplementary Table 1

Associations between harsh parenting and hemisphere-specific amygdala and hippocampus volumes.

	Maternal Harsh Parenting (N=2090)		Paternal Harsh Parenting (N=1754)	
	ß (95%CI)	P	ß (95%CI)	P
Brain Outcomes				
Amygdala volume				
Left amygdala volume	-0.04 (-0.08; 0)	0.033	-0.03 (-0.07; 0.01)	0.141
Right amygdala volume	-0.03 (-0.07; 0)	0.067	-0.03 (-0.06; 0.01)	0.193
Hippocampus volume				
Left hippocampus volume	-0.02 (-0.06; 0.01)	0.183	-0.01 (-0.05; 0.03)	0.537
Right hippocampus volume	-0.01 (-0.05; 0.02)	0.485	0 (-0.04; 0.04)	0.844

Note. Models adjusted for: total ICV (total intracranial volume), child sex, age at brain MRI scan, maternal national origin, birth weight, prenatal smoking and alcohol consumption, family income, maternal education, marital status, maternal depressive symptoms. In paternal harsh parenting analyses, paternal education and depressive symptoms are included instead of the maternal covariates. Estimates are standardized.

Supplementary Table 2

Associations between the combined parental harsh parenting measure and the brain outcomes

		Model 1		Model 2	
	N	ß (95%CI)	P	ß (95%CI)	P
Brain Outcomes					
Global brain measures	2141				
Total gray matter volume		-0.05 (-0.08; -0.01)	0.010	-0.04 (-0.07; 0.00)	0.039
Cerebral white matter volume		-0.04 (-0.08; -0.01)	0.025	-0.03 (-0.07; 0.00)	0.075
Subcortical structures					
Amygdala volume		-0.04 (-0.07; 0)	0.037	-0.04 (-0.07; -0.01)	0.020
Hippocampus volume		-0.02 (-0.05; 0.02)	0.353	-0.02 (-0.05; 0.02)	0.349
Global DTI measures	2107				
Global FA		-0.02 (-0.06; 0.02)	0.390	-0.02 (-0.06; 0.02)	0.345
Global MD		0.01 (-0.04; 0.05)	0.753	0.01 (-0.03; 0.05)	0.663

Note. Associations between the combined parental harsh parenting measure (principal component) at age 3 years and child brain outcomes at age 10 years. Amygdala and hippocampal volumes averaged over both hemispheres. Model 1 adjusted for: total ICV (total intracranial volume), child age at brain MRI scan, child sex and maternal national origin. Model 2 additionally adjusted for birth weight, prenatal smoking and alcohol consumption, family income, maternal education, and paternal education, marital status and maternal and paternal depressive symptoms. Global brain measures are not adjusted for total ICV. Estimates are standardized.

Supplementary Table 3.

Associations between maternal harsh parenting and the child brain outcomes after adjustment for other adverse experiences

		Model 2		Model 3		Model 4	
	N	ß (95%CI)	P	ß (95%CI)	P	ß (95%CI)	P
Brain Outcomes							
Global brain measures							
Total gray matter volume	2090	-0.05 (-0.08; -0.01)	0.006	-0.05 (-0.09; -0.01)	0.006	-0.05 (-0.08; -0.01)	0.007
Cerebral white matter volume	2090	-0.04 (-0.08; 0)	0.035	-0.04 (-0.08; 0)	0.035	-0.04 (-0.08; 0)	0.036
Subcortical structures							
Amygdala volume	2090	-0.04 (-0.07; 0)	0.028	-0.04 (-0.07; 0)	0.026	-0.04 (-0.07; 0)	0.029

Note. Amygdala volumes averaged over both hemispheres. Model 2 adjusted for: total ICV (total intracranial volume), child age at brain MRI scan, child sex, maternal national origin, birth weight, prenatal smoking and alcohol consumption, family income, maternal education, marital status and maternal depressive symptoms. Model 3 is Model 2 additionally adjusted for marital problems at age 3 years. Model 4 is Model 2 additionally adjusted for maternal alcohol drinking problems (regular drinking problems and binge drinking) at age 5 years. Global brain measures are not adjusted for total ICV. Estimates are standardized.

Supplemental Material

Image Processing

The FreeSurfer processed T_1-weighted images and cortical surface reconstructions were visually examined for quality and inaccurate scans were excluded from subsequent analyses (Muetzel et al., 2018; Muetzel et al., 2019). The accuracy was evaluated by comparing the white and pial surface representations against the brain image at several slices and in the axial, coronal and sagittal planes, as well as viewing the 3-dimensional inflated and pial surface representations for artifact (Muetzel et al., 2018). All scans rated as unusable were excluded from analyses.

The quality of the diffusion-weighted images was inspected by manual and automated methods. The slice-wise variation of the signal was automatically examined by the DTIPrep tool (https://www.nitrc.org/projects/dtiprep/) for the presence of artifacts. Then, the voxel-wise maps of the sum-of-squares error of the diffusion tensor fit calculations were visually inspected. The signal of artifacts was rated as none, mild, moderate or severe. Cases were excluded by the automated inspection, or if they had a "severe" score in the manual ratings. The quality of the tractography data was also evaluated, first inspecting the non-linear registration to standard space and second, evaluating whether the connectivity distributions had grossly misclassified voxels (Muetzel et al., 2018).

Harsh Parenting Assessment

Mothers and fathers separately rated their discipline tactics during the past 2 weeks on a 6-point frequency scale (*Never, once, two times, three times, four times, more than four times*) with the following 10 items (Jansen et al., 2012):

1. I explained why something is wrong.
2. I sent my child to the hall or to his/her room.
3. I gave my child something else to do instead of what he/she was doing wrong.
4. **I shook my child.**
5. **I shouted or screamed angrily at my child.**
6. **I called my child names.**
7. **I threatened to give a slap but I didn't do it.**
8. I punished my child by forbidding something that he/she wanted to do or have.
9. **I angrily pinched my child's arm.**
10. **I called my child stupid or lazy or something like that.**

The assessment was based on the Parent-Child Conflict Tactics Scale (Straus et al., 1998) and as described by Jansen et al. (2012), six items (in bold) were selected based on factor analysis to be included in the harsh parenting score. A harsh parenting score was

separately computed for mothers (mean=2.88, SD=3.15) and for fathers (mean=2.22, SD=2.67), with higher scores representing greater harsh discipline use.

Additional Information on Covariates

Marital status was categorized as: "married or living with a partner" and "being single". National origin was classified into Dutch, non-Dutch Western and non-Western according to Statistics Netherlands (2004). Prenatal smoking included three categories: "never smoked", "smoked until pregnancy was known", and "continued smoking during pregnancy". Prenatal alcohol use was classified into "never during pregnancy", "until pregnancy was known", "continued drinking occasionally", and "continued drinking frequently". Maternal and paternal education, assessed in pregnancy and at age 3 years, were classified based on the highest level of education *ever reported* into "low (primary and high school and low vocational training)", "medium (university bachelor and high vocational training)" and "high education (further education)". Family income, defined as the household's net income, was reported in 10 categories ranging from "less than 450 euro per month" to "more than 4000 euro per month".

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4

Prenatal and Childhood Adverse Events and Child Brain Morphology: A Population-Based Study

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ABSTRACT

Prenatal and childhood adverse events have been shown to be related to children's cognitive and psychological development. However, the influence of early-life adversities on child brain morphology is not well understood and most studies are based on small samples and often examine only one adversity. Thus, the goal of our study is to examine the relationship between cumulative exposures to prenatal and childhood adversities and brain morphology in a large population-based study. Participants included 2,993 children from the Generation R Study, a cohort of children growing up in Rotterdam, the Netherlands. Recruitment was initiated between 2002 and 2006 and the study is currently performing the 17-19 year follow-up wave. Prenatal adversities were reported by mothers at 20-25 weeks of pregnancy and the child's lifetime exposure to adversities was reported by mothers when the children were 10 years-of-age. The total brain, grey and white matter volumes and the volume of the cerebellum, amygdala and hippocampus were assessed with magnetic resonance imaging when children were 10 years old. In total, 36% of children had mothers who were exposed to at least one adversity during pregnancy and 35% of children were exposed to adversities in childhood. In our study sample, the cumulative number of prenatal adversities was not related to any brain outcome. In contrast, per each additional childhood adverse event, the total brain volume was 0.07 standard deviations smaller (SE = 0.02, p = 0.001), with differences in both grey and white matter volumes. Childhood adversities were not related to the amygdala or hippocampal volumes. Additionally, the link between childhood events and the preadolescent brain was not modified by prenatal events and was not explained by maternal psychopathology. Our results suggest that childhood adversities, but not prenatal adverse events, are associated with smaller global brain volumes in preadolescence. Notably, this is the first large population-based study to prospectively assess the association between the cumulative number of prenatal adversities and the preadolescent brain morphology. The study findings extend the evidence from high-risk samples, providing support for a link between cumulative childhood adverse events and brain morphology in children from the general population.

INTRODUCTION

Adversities, defined as the negative experiences that deviate from the expectable environment, need to be chronic (e.g. parental loss), or sufficiently severe to require a considerable psychobiological adaptation (McLaughlin et al., 2019). Children whose mothers experienced adversities during pregnancy tend to have more behavioral problems (Jones et al., 2019) and childhood adversities are associated with poorer intellectual performance (Nelson III et al., 2007). Although studies in high-risk samples have addressed the relation between early-life adversity and child brain morphology (McLaughlin et al., 2019), the association of prenatal and childhood adversities with child brain morphology is not well documented in the general population.

Fetal life, when the brain undergoes its greatest relative growth, is a critical period for brain development (Davis & Narayan, 2020). Starting with differentiation of the ectoderm into neural tissue, there is a complex cascade of events that involve neurulation, neurogenesis and subsequent migration, apoptosis, synaptogenesis and dendritic arborization (Davis & Narayan, 2020; Stiles & Jernigan, 2010). This developmental period of incredible growth and change is a sensitive window, in which environmental factors that generate maternal toxic psychological stress may have profound and lasting effects (Nelson, 2020). However, few studies have examined the relation between prenatal adversities and offspring neurodevelopment. As reviewed by Franke et al. (2020), studies examining head circumference (HC) at birth showed mixed results. For example, prenatal adversities were not related to HC at birth in a population-based sample (N=4,211) (Obel et al., 2003), whereas a small *positive* association was found in a larger cohort (N=78,017) (Tegethoff et al., 2010). HC metrics are easily accessible and a proxy for total brain volume. However, they might not capture region-specific differences (Franke et al., 2020). Only one study assessed prenatal adversities and child brain morphology using magnetic resonance imaging (MRI) and found that girls whose mothers were exposed to an adverse event in pregnancy had *larger* amygdala volumes (N=68) (Jones et al., 2019). To date, no large population-based study has examined the relation between cumulative prenatal adversities and child brain morphology.

In contrast, there is substantial research on childhood adversities and offspring neurodevelopment, including case-control studies, where adversities are often severe (e.g. institutionalization), and studies in children exposed to a more graded scale of events. Severe adversities have been related to smaller cerebellar (McLaughlin et al., 2019) and global brain volumes, with differences in multiple brain regions (Bick & Nelson, 2016). Evidence for differences in the amygdala and hippocampus is mixed, with both larger (Roth et al., 2018; Tupler & De Bellis, 2006) and smaller volumes (Hanson et al., 2015) reported. Hanson et al. (2015) examined three samples of children exposed to different

adversities (physical abuse, neglect, low socioeconomic status (SES)) and found smaller amygdala in relation to all adversities.

Studies in children exposed to more common adversities have reported differences in the cerebellum, cortex and limbic structures. Cumulative early-life adverse experiences were associated with smaller grey matter volumes of the cerebellum, the amygdala, and multiple cortical regions in the frontal, parietal, and temporal lobes in a sample of 58 adolescents (Walsh et al., 2014) and with smaller prefrontal cortex, amygdala and hippocampal volumes in a study oversampled for child depression (Luby et al., 2017; Luby et al., 2019). Importantly, the adversity definition in the latter study included parental psychopathology. Although having a parent with psychopathology may represent an adversity, shared genetic factors may underlie the association (Franke et al., 2020) and parental psychopathology may additionally interact with the adversities' effect (Bergink et al., 2016).

There are also other relevant factors that may influence the association between early adverse events and downstream brain morphology. First, socioeconomic status (SES) is related to child brain morphology and function, possibly through factors such as exposure to pollution, and the availability of education, cognitive stimulation, and healthcare (Olson et al., 2021). Importantly, while adversity occurs more often in individuals experiencing poverty, stress and the consequences thereof may also occur in other socioeconomic strata. The effects of adversity are likely explained by the biological stress response (Amso & Lynn, 2017), thus suggesting that adversity and SES could have independent pathways underlying their effects on brain morphology. Determining whether early-life adversity is associated with brain morphological differences independent of the already known effect of SES is important to obtain a more precise estimation of the role of adversity on the brain (Amso & Lynn, 2017). Second, accounting for the potential direct neurobiological effect of maternal smoking and alcohol use during pregnancy (Mick et al., 2002) can help to elucidate whether childhood adversity is related to the child's brain, independent of these exposures.

Evidence suggests a cumulative relation between childhood adversities and numerous health-related outcomes, including health-risk behaviors and psychiatric disorders (Felitti et al., 1998). To address a potential cumulative adversity effect on brain morphology, two main approaches have been proposed. First, the "lumping" approach focuses on the cumulative number of adverse events, assuming that different stressful events have similar effects on brain morphology (Smith & Pollak, 2020). Second, the "dimensional" approach, proposed by McLaughlin and Sheridan (2016), distinguishes between threatening events such as community violence and physical abuse, and deprivation-related events, or those related to lack of cognitive and social stimulation such as neglect and poverty. The dimensional approach hypothesizes potentially different psychobiological effects and underlying mechanisms between the two groups (McLaughlin & Sheridan,

2016). However, largely similar brain differences have been described across the exposure to threatening and to deprivation-related events (Bick & Nelson, 2016; Hanson et al., 2015; Smith & Pollak, 2020), suggesting low specificity across adversity types. We acknowledge that both approaches could offer a complementary perspective on the mechanisms and public health implications of childhood adversity, and the debate on how to assess adversity is still an open question. It is however clear that compared to examining single adversities, the cumulative adversity assessment offers a more naturalistic view of the adversity exposure, because adverse events are often related and tend to co-occur (Smith & Pollak, 2020). In the current study, we assessed the association between early-life adversities and brain morphology based on the broader cumulative adversity approach.

Notably, a randomized-controlled trial in institutionalized children demonstrated that cognitive outcomes improved when children were placed into foster care, especially if this placement occurred at younger ages (Nelson III et al., 2007). Sheridan et al. (2012) additionally described white matter volume differences between the children who remained in the institution and those never institutionalized, but not when comparing the foster care group with the never-institutionalized group. Thus, child neurodevelopment can improve, within the available biological reserve, after adversity ceases (White, 2019). This has two implications for our study. First, the timing of adversity exposure may influence the association with brain morphology. Children with no childhood adversities, but whose mothers experienced adversities during pregnancy may show differences due to the pronounced neurodevelopment that occurs during prenatal life (White, 2019). Children with adversities in both the prenatal and childhood periods may have the largest brain differences. Thus, we examined adversities in both periods in relation to child brain morphology. Second, when adversity occurs only prenatally, delays in brain development could "catch-up" postnatally, approaching the typical growth curve (White, 2019). To examine whether postnatal brain changes could have a role in our association of interest, we included fetal HC measures in sensitivity analyses.

Overall, evidence suggests that childhood adversity may be associated with the volume of the amygdala, the hippocampus and the cerebellum (Edmiston et al., 2011; McLaughlin et al., 2019; Walsh et al., 2014). Adversity has also been found to be associated with widespread cortical differences, including the frontal, parietal, temporal, and occipital lobes (Bick & Nelson, 2016; Edmiston et al., 2011; McLaughlin et al., 2014; Walsh et al., 2014), likely indicating a global cortical effect of adversity. Thus, in this population-based study, we examined the relationship between cumulative prenatal and childhood adversities and preadolescent brain morphology, with a focus on the hippocampus, amygdala, cerebellum and global brain volumes. We hypothesized a greater number of adversities would be related to smaller global brain, amygdala and hippocampal volumes. We additionally hypothesized a stronger association between

childhood adversities and brain morphology in children whose mothers were exposed to prenatal adversities.

MATERIALS AND METHODS

Participants

This study is part of the Generation R Study, a population-based prenatal birth cohort in Rotterdam, the Netherlands (Jaddoe et al., 2012). In total, 9,778 pregnant mothers with a delivery date from April 2002 to January 2006 were enrolled, and information was collected from children and parents by questionnaires, interviews and research visits. Study protocols for each wave of data collection were approved by the Medical Ethical Committee of the Erasmus Medical Center and all parents gave written informed consent.

T_1-weighted MRI scans were acquired in 3,966 9-to-11-year-old children (White et al., 2018), of which 3,186 had good image quality data. Among these children, 3,146 had complete information on prenatal and/or childhood adversities. We randomly excluded one sibling (N=153) to avoid non-independent data. In total, 2,993 children were included in analyses (2,242 in prenatal adversities analyses and 2,923 in childhood adversities analyses; Figure S1).

Measures

Adversities

Prenatal adversities.

Adverse events occurring prenatally and shortly before pregnancy were assessed with a Dutch-adapted version of the Social Readjustment Rating Scale (SRRS)(Miller & Rahe, 1997). At 20-25 weeks of pregnancy, mothers reported the occurrence of ten stressful events in the preceding 12 months (e.g. serious illnesses of family members, partner's death) (Molenaar et al., 2019). As part of the adversity score, we included a measure of substantial financial downturn, to assess instability and drastic changes in the pre-existing social and economic resources that could have led to a prolonged or severe biological stress response. The occurrence of robbery, theft, physical abuse or rape was self-reported by the participant as a response to a single question, and was additionally included in the prenatal adversities measure, given the relevance of these adverse experiences. Moving to a new home, originally assessed by the SRRS, was excluded as it could also reflect a positive situation. A '*prenatal adversities score*' was computed as the cumulative number of occurrences of ten adverse events (Table S1).

Childhood adversities.

Occurrence of stressful life events from birth to age 10 years was reported by mothers during an interview when children were 10 years old (Dunn et al., 2019). This instrument was based on the TRAILS study questionnaires (Amone-P'Olak et al., 2009) and the Life Events and Difficulty Schedule (Brown & Harris, 1978), and comprised twenty-four events of varying severity (e.g. high amount of school work, parental conflicts). To better measure *severe* adversities in this population-based sample, specific adverse events were selected using as reference the ACEs studies (e.g. Felitti et al. (1998)). A '*childhood adversities score*' was computed as the cumulative occurrence of these adversities (Table S2).

The measures of prenatal and childhood adversities were defined assuming equal weights of the individual events, following the "cumulative" mainstream approach to adversity, as outlined by Smith and Pollak (2020). This approach provides a useful measure of adversity, which is simple and can be replicated across studies independent of sample-specific differences that otherwise affect data-driven approaches (e.g. latent constructs).

Brain Imaging

Brain MRI data were obtained in 9-11-year-old children using a 3 Tesla GE 750w Discovery platform (General Electric, Milwaukee, WI)(White et al., 2018). T_1-weighted images were collected with a receive-only 8-channel head coil and an inversion recovery fast spoil gradient recalled sequence (TR=8.77ms, TE=3.4ms, TI=600ms, Flip angle=10°, Field of view=220x220, Acquisition matrix=220x220, Slice thickness=1mm, Number of slices=230, ARC acceleration factor=2).

We processed and conducted the segmentation and reconstruction of the neuroimaging data with the FreeSurfer image analysis suite (v.6.0)(Fischl, 2012). Reconstructed images were inspected for quality and poor quality reconstructions were excluded from further analyses (Supplemental Information) (Muetzel et al., 2018). The total brain volume, the cortical grey and cerebral white matter volumes, the cerebellar volume, and the amygdala and hippocampal volumes were included in analyses.

Ultrasound measures

Fetal ultrasound measures were collected at three time-points during pregnancy (Henrichs et al., 2010), at a median gestational age of 13.1 weeks (95% range = 9.3, 17.5) for the first assessment, 20.5 weeks (95% range = 18.4, 23.3) for the mid-pregnancy assessment, and 30.4 weeks (95% range = 27.9, 33.0) for the last assessment (Jaddoe et al., 2007). The HC data collection was described in detail by Verburg, Steegers, et al. (2008). Briefly, sonographers established the gestational age based on the first ultrasound assessment and measured fetal HC based on the outline of the skull and to the nearest millimeter

using standardized techniques. The HC measures collected during the third trimester of pregnancy were included in the sensitivity analyses. These HC metrics have been shown to be predicted by maternal smoking during pregnancy (Jaddoe et al., 2007) and by maternal education levels (Silva et al., 2010). Additionally, the HC metrics in our sample had a correlation of 0.55 ($p < 0.001$) with the gestational age at the ultrasound assessment and of 0.38 ($p < 0.001$) with the total brain volume at age 10 years, supporting the validity of our measures. There was high reliability for the HC metrics in early pregnancy, with intra- and inter-observer intraclass correlation coefficient (ICC) of 0.995 and 0.988, respectively, and intra- and inter-observer coefficient of variation (CV) of 2.2 and 3.8, respectively (Verburg, Mulder, et al., 2008).

Covariates

We included as covariates child sex and age at the MRI scan, total intracranial volume, maternal national origin, highest household education and maternal prenatal alcohol use and smoking. Child sex was collected from birth records. Maternal national origin was defined based on her parents' birth country and was self-reported during pregnancy. Maternal national origin was categorized as Dutch, non-Dutch Western and non-Western. Mothers were considered of Dutch origin if both of her parents were born in the Netherlands. When one of her parents was born abroad, maternal origin was defined based on the country of birth of this parent. We grouped the national origin minorities as non-Dutch Western (including European, Indonesian, Japanese, Oceanian, and North American) and non-Western (including other national origins, e.g. Surinamese and Moroccan) (*Statistical Yearbook of the Netherlands 2004*, 2004) (See also: Troe et al. (2007)). The highest household education, and prenatal alcohol consumption and smoking were reported through questionnaires during pregnancy (See Supplemental Information).

Maternal psychopathology in pregnancy was assessed with the Brief Symptom Inventory, a validated and widely-used questionnaire (Derogatis, 1993). We used the global severity index score, a measure of the global severity of psychopathology, in additional analyses.

Statistical Analyses

We examined the associations of prenatal and childhood adversities with the brain outcomes using multiple linear regression. We first fitted a minimally adjusted model controlling for child sex and age at MRI scan, total intracranial volume (in amygdala and hippocampus analyses) and maternal national origin. Child sex and age at MRI scan were included as precision variables to account for typical differences in brain morphological characteristics (Lenroot & Giedd, 2006). Child intracranial volume was included in all analyses of the amygdala and hippocampus to determine whether childhood adversity was associated with the volume of these regions of interest independently of the

adversity-related global brain differences. Considering the multi-ethnic nature of our study sample, maternal national origin was controlled for to account for differences in the adversity exposure and possible anatomical brain variations across national origins (Cheng et al., 2016). In a second model, we adjusted for the highest household education as an indicator of SES. Although adversity occurs more frequently in families experiencing poverty, it is argued that both factors have an independent effect and potentially different biological mechanisms (Amso & Lynn, 2017). Therefore, we aimed to determine the association between adversity and brain morphology in children living in any socioeconomic status. Finally, we also controlled for prenatal alcohol use and smoking in a third, fully-adjusted model, since these factors may have a direct neurobiological effect (Mick et al., 2002) and could be also considered part of the pathway between prenatal adversities and brain morphology.

We subsequently examined the interaction between prenatal and childhood adversities in relation to brain morphology. Additionally, for descriptive purposes, we assessed the relation between a categorical adversity measure and the brain outcomes, using four groups: children with one or more of the prenatal adversities that we measured (N=460), children with one or more of the childhood adversities that we measured (N=433), children with adversities in both periods (N=321), and children with none of these adversities (N=958).

Several sensitivity analyses were performed. We first examined whether child sex modified the associations between adversity and brain morphology. Second, we analyzed the associations of adversity and brain morphology in a more homogeneous group, children whose mothers had a Dutch national origin, and we explored the interaction between national origin and adversity on the brain outcomes by adding an interaction term in a model that included participants from all national origin groups. Third, we explored whether associations between adversity and brain morphology were explained by maternal psychopathology, and we examined the interaction between maternal psychopathology and adversity in relation to child brain morphology. Finally, we explored whether postnatal brain growth and volumetric changes in response to environmental factors (White, 2019) could influence the association of adversity and brain morphology by assessing whether prenatal adversities were associated with HC at the last pregnancy trimester, as HC is a proxy for an early measure of total brain volume (analyses adjusted for gestational age at ultrasound).

Analyses were performed in R v.3.6.1 (R Core Team, 2020). Outcomes were standardized. Multiple imputation of missing values (maximum missingness: maternal psychopathology=23.4%) was performed ("mice" package (van Buuren & Groothuis-Oudshoorn, 2011)), and results were pooled across 25 imputed datasets. We found no signs of violation of the regression assumptions (i.e. independence, normal distribution, homoscedasticity). Additionally, the variance inflation factor was < 2.5 for all variables

in analyses of the interaction between prenatal and childhood adversity, suggesting no multicollinearity. Adjustment for multiple testing was performed using the Bonferroni approach in the analyses with prenatal adversities, childhood adversities and the interaction between prenatal and childhood adversities (15 tests, including all brain outcomes, except for total brain volume).

Non-response and MRI exclusions analyses

Children included in the analyses of prenatal adversities and brain morphology (N=2,242) were compared to children with data on prenatal adversities but no neuroimaging data available (N=3,552). Continuous variables were compared with the Mann-Whitney U test and categorical variables with chi-squared tests. Mothers of children without imaging data were more often exposed to prenatal adversities (one or more events: 40.7%) than those of children in analyses (one or more events: 36.1%) and were less often highly educated (22.1% vs 30.5%). Additionally, mothers of children without imaging data were less often from Dutch origins (No imaging data group: 50.6%; Study sample: 61.1%) and had more psychiatric symptoms (median (IQR)=0.19 (0.1, 0.4)) than those in analyses (median (IQR)=0.15 (0.1, 0.3)).

Children with prenatal and/or childhood adversity and neuroimaging data available but who were excluded due to non-usable MRI data (N = 760) did not differ from children included in analyses (N = 2993) in the exposure to prenatal ($p = 0.27$) or childhood adversities ($p = 0.31$), in maternal national origins ($p = 0.09$) or in maternal psychiatric symptoms ($p = 0.26$). Excluded children more often had mothers with lower education (54.0%) compared to those in analyses (47.3%; $p = 0.01$).

RESULTS

In our study sample, the child age at the MRI scan was between 8.72 - 11.99 years (median: 9.93 years), with 90% of children below the age of 11.19 years. In total, 36% of children had mothers who were exposed to at least one prenatal adversity and 35% of children were exposed to adversities during childhood (Table 1). Children with mothers exposed to prenatal adversities were more likely to experience adversities during childhood (41%) compared to those without prenatal adversities (31%).The most commonly reported prenatal event was a substantial financial downturn (14.5%), followed by a serious illness of a family member (11.6%)(Table S1). In childhood, parental separation or divorce was the most prevalent event (21.45%)(Table S2). Distributions and Pearson correlations for all variables of interest are presented in Figure S2 and Table S3, respectively. There was a correlation of 0.13 ($p < 0.001$) between prenatal and childhood adversities. Prenatal and childhood adversities were more common in children of non-Western

mothers (any adversity = 51.4%, and 43.7%, respectively) compared to children of Dutch mothers (any adversity = 30.2% and 31.1%, respectively). Prenatal adversities occurred in 37.0% of boys, and 35.0% of girls, and childhood adversities in 36.3% of boys and 33.3% of girls.

Table 1. Baseline characteristics

	mean (SD) or %*	N
Adversity measures		
Prenatal adversities (10 items), % (N=2242)		
0	63.9	1432
1	20.6	461
2	10.5	236
3	3.8	85
4 or more	1.2	28
Childhood adversities (4 items), % (N=2923)		
0	64.9	1897
1	27.2	795
2	6.3	185
3	1.4	41
4	0.2	5
Child characteristics		
Sex, % girls	50.8	1521
Age at MRI scan, years	10.1 (0.6)	2993
Parental Characteristics		
Maternal national origin, %		2993
Dutch	57.6	1725
Non-Western	30.3	906
Other Western	12.1	362
Highest household education, %		2993
Low education	41.0	1227
Medium education	22.4	670
High education	36.6	1096
Maternal prenatal alcohol use, % never during pregnancy	41.0	1226
Maternal prenatal smoking, % never during pregnancy	76.9	2303
Maternal Psychiatric Symptoms, median (Q1,Q3)	0.15 (0.06, 0.32)	2993

Characteristics of the sample with information for **prenatal AND/OR childhood adversities** and brain structural MRI data (N=2993). *Otherwise indicated. Based on imputed datasets.

The cumulative number of prenatal adverse events was not related to any brain outcome (Table 2). In contrast, a consistent association was found between childhood adversities and all global brain metrics (total brain, cortical grey and white matter vol-

umes and total cerebellar volumes). Children had, on average, a 0.11 standard-deviation smaller total brain volume (SE=0.02, p<0.001) per each additional childhood adverse event, adjusting for child sex, age at the MRI scan, and maternal national origin. The associations between childhood adversities and the total brain, cortical grey and white matter volumes remained after adjustment for parental education, and prenatal alcohol use and smoking (Total brain volume: B=-0.07, SE=0.02, p=0.001) (Figure S3). Childhood adversities were not related to the amygdala and hippocampus (Table 2). After adjustment for multiple testing, the associations between childhood adversities and the cortical grey (p-adjusted<0.05), and cerebral white matter volumes (p-adjusted=0.03) remained.

Table 2. Associations between cumulative prenatal and childhood adversities and child brain morphology

	Model 1			Model 2			Model 3		
	B	SE	P	B	SE	P	B	SE	P
Prenatal adversities									
Global brain metrics									
Total brain volume	-0.03	0.02	0.14	-0.02	0.02	0.39	-0.01	0.02	0.52
Cortical grey matter volume	-0.03	0.02	0.20	-0.01	0.02	0.57	-0.01	0.02	0.71
Cerebral white matter volume	-0.02	0.02	0.23	-0.02	0.02	0.41	-0.01	0.02	0.56
Total cerebellar volume	-0.03	0.02	0.10	-0.03	0.02	0.20	-0.02	0.02	0.26
Subcortical brain metrics									
Amygdala, mean volume	0.02	0.02	0.40	0.01	0.02	0.41	0.01	0.02	0.52
Hippocampus, mean volume	0.01	0.02	0.42	0.01	0.02	0.42	0.01	0.02	0.50
Childhood adversities									
Global brain metrics									
Total brain volume	-0.11	0.02	<0.001	-0.08	0.02	<0.001	-0.07	0.02	0.001
Cortical grey matter volume	-0.11	0.02	<0.001	-0.08	0.02	0.001	-0.07	0.02	0.003*
Cerebral white matter volume	-0.10	0.02	<0.001	-0.08	0.02	0.001	-0.07	0.02	0.002*
Total cerebellar volume	-0.07	0.02	0.003	-0.05	0.02	0.03	-0.05	0.02	0.06
Subcortical brain metrics									
Amygdala, mean volume	0	0.02	0.90	0	0.02	0.87	-0.01	0.02	0.70
Hippocampus, mean volume	-0.01	0.02	0.58	-0.01	0.02	0.63	-0.01	0.02	0.59

Model 1 is adjusted for child age at MRI scan, child sex, total intracranial volume (in subcortical metrics), and maternal national origin. Model 2 is additionally adjusted for the highest household education. Model 3 is additionally adjusted for maternal prenatal alcohol use and smoking. All outcomes are standardized. N=2242 in prenatal adversities analyses, N=2923 in childhood adversities analyses. *These p-values survived correction for multiple testing.

No interaction was observed between prenatal and childhood adversities in relation to child brain morphology (Table 3). Also, when using the categorical adversity measure, the exposure to *only* prenatal adversities was not related to the total brain

volume, whereas the specific exposure to childhood adversities was associated with a 0.10 standard-deviation smaller total brain volume (p=0.04). Additionally, children with adversities in both periods had a 0.10 standard-deviation smaller total brain volume than those non-exposed to any of the adversities measured (p=0.06). Altogether, our results suggest that only childhood events are related to brain morphology and that this association is independent of the occurrence of prenatal adversities (Figure 1).

Table 3. Interaction between prenatal adversities and adversities in childhood in relation to brain morphology

	Main effect: Prenatal adversities			Main effect: Adversities in childhood			Interaction Effect		
	B	SE	P	B	SE	P	B	SE	P
Global metrics									
Total brain volume	-0.02	0.02	0.33	-0.10	0.03	0.002	0.04	0.03	0.15
Cortical grey matter volume	-0.02	0.02	0.33	-0.10	0.03	0.001	0.04	0.03	0.08
Cerebral white matter volume	-0.02	0.03	0.55	-0.09	0.03	0.01	0.02	0.03	0.40
Total cerebellar volume	-0.03	0.03	0.22	-0.06	0.03	0.09	0.03	0.03	0.35
Subcortical metrics									
Amygdala, mean volume	0	0.02	0.96	-0.02	0.03	0.43	0.03	0.02	0.24
Hippocampus, mean volume	0.01	0.02	0.56	0.01	0.03	0.69	0	0.02	0.94

Model is adjusted for child age at MRI scan, child sex, total intracranial volume (in subcortical metrics), maternal national origin, the highest education in the household, maternal prenatal alcohol use and maternal prenatal smoking. All brain outcomes were standardized. Adversities measures represent the cumulative number of events. N=2172

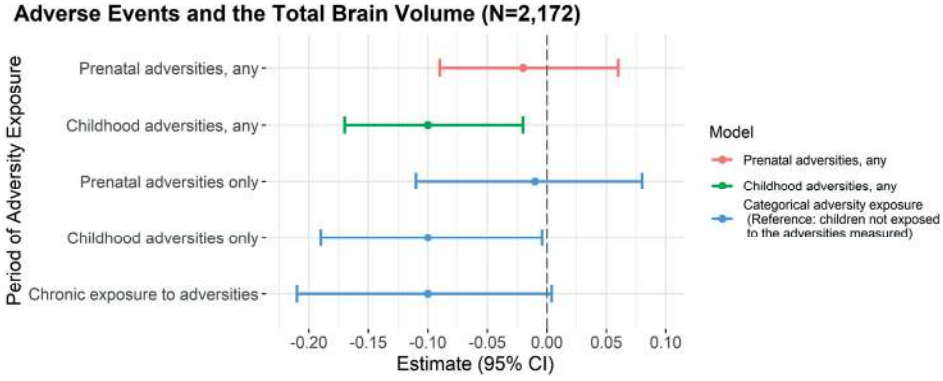

Figure 1. Associations between prenatal and childhood adversities with the total brain volume.

We further examined the specificity and robustness of the association between childhood adversities and brain morphology. No interaction was found between child sex and childhood adversities for any brain outcome. When including only children with

Dutch mothers, childhood adversities were related to the total brain, grey and white matter, and cerebellar volumes (Table S4) and there was no evidence of a significant moderating effect of national origin on the association between adversities and brain morphology. Also, the associations between childhood adversities and brain morphology were not explained nor modified by maternal prenatal psychopathology (Table S5). Additionally, the cumulative number of prenatal adversities was not related to variations in the fetal HC (B=0.00, SE=0.02, p=0.82; N=2,168). Finally, we performed a post-hoc analysis to assess whether the global brain differences observed in relation to childhood adversities were driven by a specific adversity. We found that, except for psychological abuse (B = 0.00, SE = 0.05, p = 0.94), all childhood adversities were similarly related to total brain volume (e.g. parental loss: B = -0.11, SE = 0.04, p = 0.004), supporting the validity of our cumulative approach.

DISCUSSION

In this population-based study, childhood adversities, but not prenatal adverse events experienced by the mother, were related to global brain volume differences at age 10 years. Our study provides two novel contributions to the literature. This is the first study to examine the association between cumulative prenatal adversities and brain structure in children from the general population. Contrary to our hypothesis, we found no relationship between cumulative prenatal adversities and preadolescent brain morphology using a large population-based sample, an assessment of prenatal adversities while mothers were pregnant and neuroimaging data. Second, cumulative childhood adversities were related to smaller total brain volumes and differences were observed across grey and white matter volumes. These findings are consistent with research in some small high-risk samples, supporting a relation between cumulative childhood adversities and child neurodevelopment.

The absence of associations between prenatal adversities and child brain morphology is surprising, as the brain undergoes dramatic developmental changes during pregnancy (White, 2019). Our study may have lacked sufficient power to observe subtle effects. However, we assessed a considerably larger sample than previous studies (Jones et al., 2019). The brain can adapt in response to environmental effects (Bick & Nelson, 2016), which raises the question of whether brain postnatal volumetric changes could have obscured an association between prenatal adversities and brain morphology. Given a rich and positive childhood environment, the brain development of children whose mothers experienced stress in pregnancy could catch-up and return to the normative trajectory (White, 2019). If this were the case, prenatal adversities would be related to brain differences earlier in life. However, we found no association between

prenatal events and HC in the last pregnancy trimester, arguing against the plasticity hypothesis (White, 2019) (see also a study from this cohort examining family dysfunction and fetal HC (Henrichs et al., 2010)). It is also possible that the adversity type and severity influence the relation with brain morphology. Whereas Jones et al. (2019) found a relation between the gestational exposure to a natural disaster and amygdala volumes, the cumulative exposure to a range of more normative adverse events was not associated with the global brain volume nor the amygdala and hippocampus in our study.

Numerous studies have examined *childhood* adversity and brain morphology, but results are difficult to compare due to differences in the events assessed, the age of occurrence of adversities and the age at the MRI assessment (Bick & Nelson, 2016). Overall, research suggests that children exposed to early-life adversity have smaller total brain, grey and white matter, and cerebellar volumes (Bick & Nelson, 2016). Consistently, we observed that childhood adversity was related to smaller total brain volumes, and this finding was robust to the adjustment for confounders. Analyses with the grey and white matter volumes further supported this association. Additionally, maternal psychopathology did not explain nor modify the relation between childhood adversity and these brain outcomes. Our results might be interpreted as reflecting a causal effect of adversity on child brain morphology, but our analyses are based on an observational study sample and a single MRI assessment, thus precluding the inference of causality (Hamaker et al., 2020). Other explanations for our findings are also possible. Importantly, genetic and biological characteristics, such as psychological traits, and genetic influences on hormonal and neural pathways, may underlie our findings. These factors are partly heritable and simultaneously related to the exposure to adversity (e.g. emotional abuse (Pittner et al., 2019)), which could explain a non-causal link between early-life adversity and child brain morphology.

Contrary to what we expected, childhood adversities were not related to the limbic volumes. The amygdala and hippocampus are of particular interest because they have a high density of cortisol receptors and cortisol influences the neuronal development (Franke et al., 2020). Interestingly, both larger and smaller amygdala and hippocampal volumes have been reported (Hanson et al., 2015; Roth et al., 2018; Tupler & De Bellis, 2006). In addition to the methodological differences across studies, various hypotheses could underlie these mixed findings. The volumetric growth of the amygdala and hippocampus peaks at around age 10 years (Uematsu et al., 2012), thus different findings could be expected between studies assessing brain morphology during childhood, preadolescence, and at later ages. The adversity severity may also influence the results, and the impact of early adversity in some structures may only become apparent later in development (Bick & Nelson, 2016). Further, the amygdala (Jhaveri et al., 2018) and hippocampus (Imayoshi et al., 2008) show continued neurogenesis after fetal life, sug-

gesting that these regions could undergo plastic changes in response to adversity and other environmental factors.

Our adversity measures were selected with a focus on *concrete* environmental events, that could generate stress in the pregnant mother or the child and require a substantial psychobiological adaptation (McLaughlin et al., 2019). The cumulative prenatal adversity measure was based on a major life events inventory (Miller & Rahe, 1997), similar to those included in other population-based studies (Jensen et al., 2018). Similarly, our childhood adversity measure included events assessed by key childhood adversities studies (Dong et al., 2004; Felitti et al., 1998), previously shown to be associated with greater child psychopathology (Dunn et al., 2019). Different items were used in the prenatal and childhood adversity measures, to focus on *maternal* stressful events in the prenatal measure, and on *childhood* adverse events in the latter measure. Consistent with previous studies (Jensen et al., 2018), the cumulative exposure to prenatal adversities was related to the number of childhood adversities. Our additive approach to adversity was based on the well-established "lumping" adversity framework (Smith & Pollak, 2020). Although multiple alternatives have been proposed to assign weights to the specific adverse events, based on factors like the severity, intensity, and the timing of occurrence (Smith & Pollak, 2020), there is no current consensus. Future studies should examine the role of these factors, and especially focus on the variability among individual perceptions of adversity, which likely has a unique influence in the determination of the adversity effects (Smith & Pollak, 2020).

Our study has some limitations. First, we did not account for the age of occurrence of childhood adversities. Although events at specific ages could have different effects in brain morphology, it is difficult to determine the exact period of occurrence of adversities that are often chronic and variable (Jones et al., 2019). Second, mothers reported childhood adversities at age 10 years and thus these reports could be affected by recall bias. Nonetheless, other methods to collect information on childhood adversity in the general population, such as adolescent reports, are limited by the accuracy in reporting early-life events (Roth et al., 2018). Third, mothers of children without imaging data were more often exposed to prenatal adversities and were less often highly educated than mothers in our study. Fourth, we did not examine national origin in detail given the limited sample size for specific groups. Additionally, we only included maternal national origin, as we expected a potentially differential exposure to prenatal adversities by the national origin of the pregnant mother in contrast to the biological father. Finally, the prenatal adversity measure was based on information collected when mothers were 20-25 weeks pregnant about adverse events that occurred in the preceding 12 months. By including events that occurred before pregnancy, we could have miss-classified some women who were not experiencing prenatal stress as exposed. However, cumulative preconception adversities have also been shown to predict poor offspring outcomes

(Witt et al., 2014). Additionally, events occurring after the 20-25-week assessment (in the third trimester of pregnancy) were not included, thus leading to a potential under-inclusion of late prenatal adversities.

CONCLUSION

In conclusion, we found that the number of adversities experienced by the mother during pregnancy was not related to brain morphological differences in children from the general population. Childhood adversities were consistently associated with smaller brain volumes, with alterations in both grey and white matter volumes. The association between childhood adversities and the global brain volume was not modified by maternal psychopathology, nor by the number of prenatal adversities. Our results support a cumulative association between childhood adversities and brain morphology, previously described in small high-risk samples. If the adversity and brain morphology relation is replicated in large samples with repeated MRI and adversity assessments, priority should be given to intervention studies that determine whether providing additional support to children following periods of adversities will prevent the emergence of brain differences.

ETHICS STATEMENT

All study protocols and the measurements assessed in each wave of data collection were approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam.

DATA AVAILABILITY

The datasets analyzed in this study are currently not publicly available due to legal and ethical restraints due to the General Data Protection Regulations (GDPR). However the consent has been altered for the current wave of data collection which will provide the participants the option to determine the extent that they want their data shared. Via data transfer agreements, the data can be made available upon request. Interested researchers can direct their requests to Vincent Jaddoe (v.jaddoe@erasmusmc.nl).

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SUPPLEMENTAL INFORMATION

Brain Imaging

Reconstructed FreeSurfer images were visually examined for accuracy as described previously (Muetzel et al., 2018; Muetzel et al., 2019). Eight trained and reliable raters compared the white and pial surfaces against the brain image at several slices and in sagittal, coronal, and axial planes, and visually inspected for artifacts in the 3-dimensional inflated and pial surface representations. All brain images were rated on a 3-point scale, and images considered of "poor" quality were excluded from analyses. To ensure inter-rater reliability, a training was initially performed with a standardized MRI set, and raters were considered reliable if they rated a training MRI set correctly. The amygdala and hippocampal segmentation was visually inspected by Weeland et al. (2021) in a subset of 2,551 MRI scans, with less than 1% of the images deemed as poor quality, suggesting a low rate of problematic amygdala and hippocampal segmentations in the present cohort study.

Covariates

Alcohol consumption during pregnancy included four categories: "never during pregnancy", "until pregnancy was known", "continued drinking occasionally in pregnancy", "continued drinking frequently in pregnancy". Maternal prenatal smoking was categorized into: "never during pregnancy", "until pregnancy was known" and "continued in pregnancy". Information on maternal and paternal education was collected by self-report during pregnancy and was classified following the Dutch standard classification of education (Statistics Netherlands, 2005). The highest education in the household was included in analyses.

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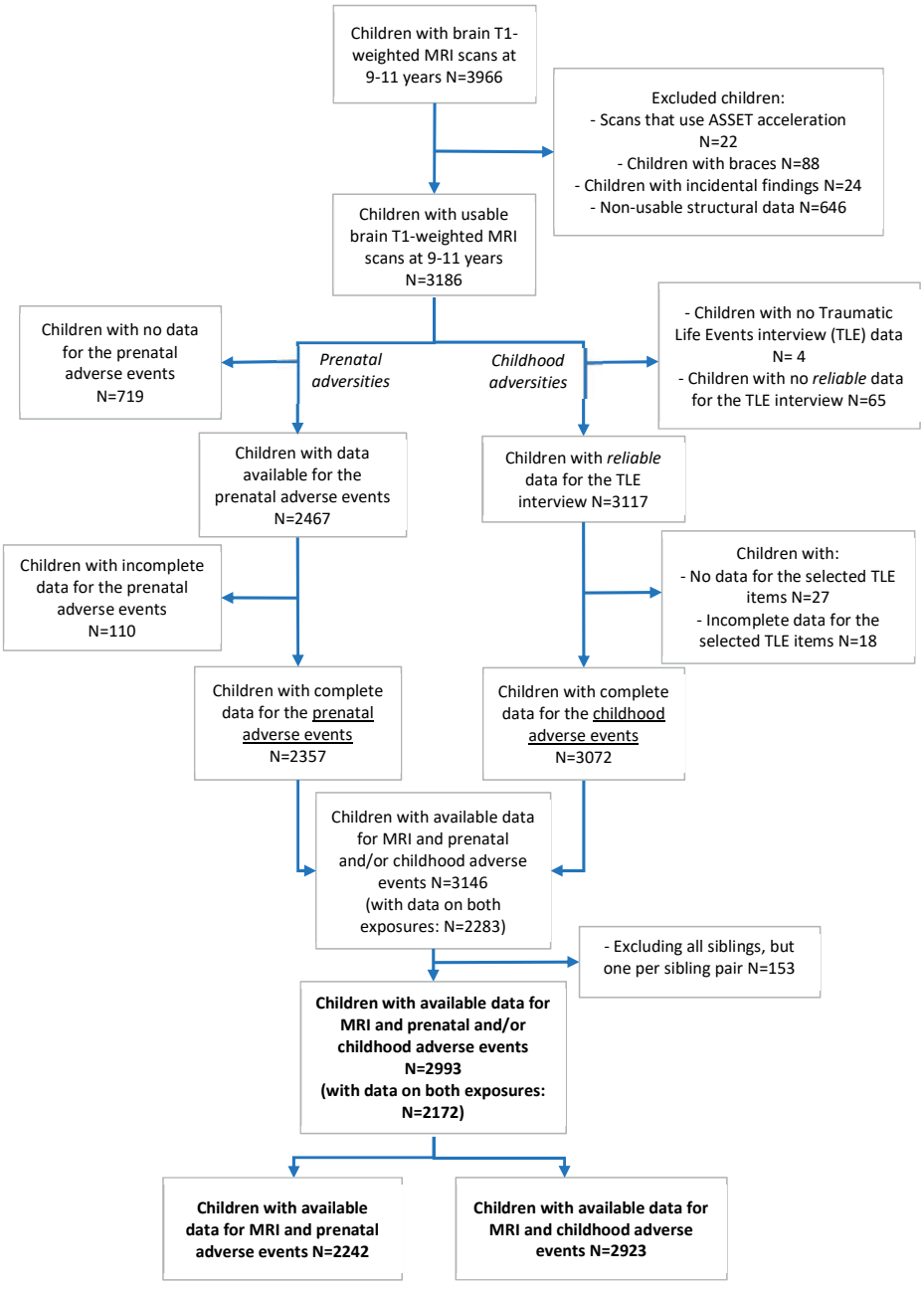
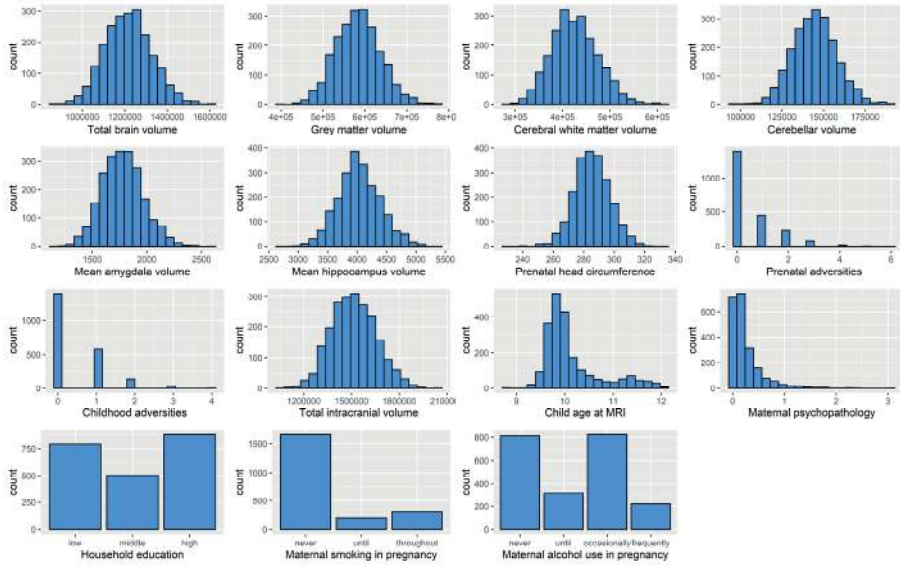
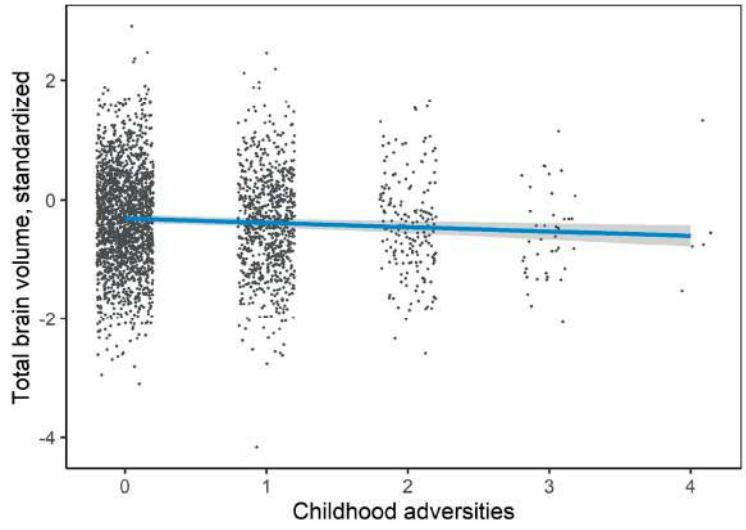


Supplementary Figure 1. Flowchart of sample selection



Supplementary Figure 2. Histograms of main variables of interest

Note. Value labels: "never" = never during pregnancy; "until" = until pregnancy was known; "throughout" = continued during pregnancy; "occasionally" = continued occasionally during pregnancy; "frequently" = continued frequently during pregnancy. Household education classified as: low (secondary, phase 2 or lower education), middle (higher, phase 1) and high (higher, phase 2) education. N = 2172.



Supplementary Figure 3. Association between childhood adversities and the total brain volume.

Note. Plot of the association between childhood adversities and the total brain volume adjusted for covariates.

Supplementary Table 1. Prevalence of prenatal adverse events

Event	Prevalence, %	N exposed
Have you been a victim of robbery, theft, physical abuse or rape?	3.88	87
Have you suffered a substantial downturn in your financial situation?	14.5	325
Have you become unemployed?	8.97	201
Has your partner or other member of your family become unemployed?	6.51	146
Has one or more of your children been seriously ill?	1.52	34
Has your partner, or other family member, or one of your parents (in-law) been seriously ill?	11.6	260
Has one of your children died?	0.71	16
Has your partner died?	0.04	1
Has your father or mother (in-law), a brother or sister, or good friend died?	7.09	159
Have you had a divorce or broken off the relationship with your partner?	3.57	80
Any category reported	36.13	810

N=2242

Supplementary Table 2. Prevalence of childhood adverse events

Category of childhood exposure	Prevalence per category, %	N exposed
Psychological abuse		
Has anyone almost used physical violence against your child? So that it did not actually happen, but your child was scared.	11.53	337
Physical abuse		
Has anyone ever used physical violence against your child? For example, beating him/her up.	6.77	198
Sexual abuse	4.41	129
Has anyone made sexual comments or movements towards your child?*	3.42	100
Did your child experience inappropriate sexual behavior?*	1.61	47
Parental loss	22.03	644
Is your child's father / mother or other caregiver still alive? (reversed)*	0.89	26
Are you and your partner divorced or separated?*	21.45	627
Any category reported	35.1	1026

N=2923. * Sexual abuse and parental loss categories include two items.

Supplementary Table 3. Correlation matrix for the main variables of interest

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Total brain volume	-															
2	Cortical grey matter volume	0.95***	-														
3	Cerebral white matter volume	0.94***	0.82***	-													
4	Total cerebellar volume	0.66***	0.56***	0.54***	-												
5	Amygdala, mean volume	0.68***	0.66***	0.62***	0.44***	-											
6	Hippocampus, mean volume	0.66***	0.62***	0.60***	0.48***	0.67***	-										
7	Prenatal head circumference	0.38***	0.37***	0.35***	0.27***	0.25***	0.26***	-									
8	Prenatal adversities	-0.06**	-0.07**	-0.05*	-0.07**	-0.02	-0.03	-0.03	-								
9	Adversities in childhood	-0.07***	-0.08***	-0.06**	-0.05*	-0.03	-0.02	-0.04	0.13***	-							
10	Total intracranial volume	0.93***	0.87***	0.89***	0.65***	0.62***	0.62***	0.40***	-0.07***	-0.06**	-						
11	Age at the MRI scan	0.05*	-0.01	0.11***	0.06**	0.05*	0.06**	-0.01	0.01	0.05*	0.08***	-					
12	Child sex	-0.51***	-0.47***	-0.48***	-0.41***	-0.40***	-0.35***	-0.22***	0	-0.04	-0.51***	-0.04*	-				
13	Highest household education	0.19***	0.22***	0.12***	0.14***	0.10***	0.11***	0.10***	-0.17***	-0.20***	0.20***	-0.06**	0	-			
14	Maternal prenatal alcohol use	0.13***	0.15***	0.09***	0.10***	0.09***	0.09***	0.03	-0.01	-0.05*	0.14***	0.01	-0.06**	0.33***	-		
15	Maternal prenatal smoking	-0.06**	-0.07**	-0.04*	-0.02	-0.01	-0.04	-0.09***	0.11***	0.13***	-0.06**	0.05*	-0.02	-0.24***	0.14***	-	
16	Maternal psychopathology	-0.09***	-0.10***	-0.06**	-0.09***	-0.01	0	-0.06*	0.34***	0.19***	-0.10***	0.07**	-0.02	-0.26***	-0.10***	0.15***	-

Correlations in the first imputed dataset. N = 2172 except for correlations with prenatal head circumference (N = 2100). Child sex: 1 = boy, 2 = girl.
Point-biserial correlations were calculated between child sex and all variables. ***P<0.001. ** P<0.01. *P<0.05

Supplementary Table 4. Association between childhood adversities and brain morphology in children with Dutch mothers

	B	SE	P
Outcome			
Global metrics			
Total brain volume	-0.09	0.03	0.004
Cortical grey matter volume	-0.08	0.03	0.02
Cerebral white matter volume	-0.09	0.03	0.01
Total cerebellar volume	-0.08	0.03	0.02
Subcortical metrics			
Amygdala, mean volume	0	0.03	0.94
Hippocampus, mean volume	-0.03	0.03	0.37

Analyses performed in children with Dutch mothers. Model adjusted for child age at MRI scan, child sex, total intracranial volume (in subcortical metrics), the highest education in the household, maternal prenatal alcohol use and maternal prenatal smoking. All outcomes are standardized. N=1669.

Supplementary Table 5. Interaction between maternal psychopathology and childhood adversities in relation to child brain morphology

	Interaction effect		
	B	SE	P
Outcome			
Global metrics			
Total brain volume	0.08	0.06	0.19
Cortical grey matter volume	0.08	0.06	0.23
Cerebral white matter volume	0.09	0.07	0.19
Total cerebellar volume	0.02	0.06	0.78
Subcortical metrics			
Amygdala, mean volume	0.01	0.06	0.91
Hippocampus, mean volume	0.02	0.06	0.74

Model adjusted for child age at MRI scan, child sex, total intracranial volume (in subcortical metrics), maternal national origin, the highest education in the household, maternal prenatal alcohol use, maternal prenatal smoking, maternal psychiatric symptoms and the interaction term of maternal psychiatric symptoms with childhood adversities. All outcomes are standardized. N=2923

5

Are all threats equal? Associations of childhood exposure to physical attack versus threatened violence with preadolescent brain structure

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ABSTRACT

Background: Neurodevelopmental studies of childhood adversity often define threatening experiences as those involving harm or the threat of harm. Whether effects differ between experiences involving harm ("physical attack") versus the threat of harm alone ("threatened violence") remains underexplored. We hypothesized that while both types of experiences would be associated with smaller preadolescent global and corticolimbic brain volumes, associations with physical attack would be greater.

Methods: Generation R Study researchers (the Netherlands) acquired T1-weighted scans from 2,905 preadolescent children, computed brain volumes using FreeSurfer, and asked mothers whether their children ever experienced physical attack (n=202) or threatened violence (n=335). Using standardized global (cortical, subcortical, white matter) and corticolimbic (amygdala, hippocampus, anterior cingulate cortex, orbitofrontal cortex) volumes, we fit confounder-adjusted models.

Results: Physical attack was associated with smaller global volumes ($\beta_{cortical}$=-0.14; 95% CI: -0.26, -0.02); $\beta_{white\ matter}$=-0.16; 95% CI: -0.28, -0.03) and possibly some corticolimbic volumes, e.g., $\beta_{amygdala/ICV\text{-}adjusted}$=-0.10 (95% CI: -0.21, 0.01). We found no evidence of associations between threatened violence and smaller volumes in any outcome; instead, such estimates were small, highly uncertain, and positive in direction.

Conclusions: Experiences of physical attack and threatened violence may have quantitively different neurodevelopmental effects. Thus, qualitative differences in threatening experiences may be neurodevelopmentally salient.

INTRODUCTION

Globally, childhood mental disorders and behavior problems impose a substantial burden on population health (Vos et al., 2020; Whiteford et al., 2013). In the United States, for example, they account for more medical spending on children ($13.9 billion in 2012) than any other condition, yet current prevention efforts are hampered by an incomplete understanding of what causes them (Bui et al., 2017; Ghandour et al., 2019; Soni, 2001). Extensive research has documented the role of childhood adversity—i.e., negative experiences that entail either harmful or inadequate input (e.g., abuse or neglect, respectively) and that require significant adaptation from a typical child—in increasing the risk of child mental disorders and behavior problems (Berens et al., 2017; Humphreys & Zeanah, 2015; McLaughlin et al., 2019; Nelson & Gabard-Durnam, 2020). Foundational research exploring mental health effects of childhood adversity generally examined either *qualitative* differences in adverse experiences (i.e., specificity models investigating one adversity at a time) or *quantitative* differences in the number of adversities a child experienced (i.e., cumulative risk models) (McLaughlin et al., 2020; Smith & Pollak, 2021). Cumulative risk models have provided valuable insight over time and continue to guide practice and policy (Lanier et al., 2018). More recently, however, investigators have proposed "dimensional" models that consider both qualitative and quantitative features of a child's adverse experiences to provide greater insight into neurobiological mechanisms mediating childhood adversity and mental disorders (McLaughlin et al., 2019, 2020).

Most prominently, Sheridan and McLaughlin (2014) proposed the dimensional model of adversity, which maintains that (1) qualitative features of adverse experiences encode multiple underlying dimensions of social experiences that have distinct neurodevelopmental effects, and (2) effects will scale based on quantitative features of the adverse experiences, e.g., the frequency and severity of a child's experience (McLaughlin et al., 2014; McLaughlin & Sheridan, 2016; Sheridan & McLaughlin, 2014). Sheridan & McLaughlin (2014) initially defined two dimensions for their model: (1) experiential deprivation, or the absence of expected cognitive and social input, and (2) threatening experiences (Sheridan & McLaughlin, 2014). Borrowing from the DSM-5 definition of "traumatic event," they defined threatening experiences as those "characterized by *actual or threatened* . . . harm to one's physical integrity" (emphasis added) (Sheridan & McLaughlin, 2014). More recently, McLaughlin, Weissman & Bitrán (2019) defined threats as "experiences involving *harm or threat of harm* to the child" (emphasis added) (McLaughlin et al., 2019). Thus, the dimensional model of adversity assumes that both (1) experiences involving harm and (2) experiences involving only the threat of harm should cause similar neurodevelopmental effects, perhaps differing only based on the frequency and severity of the experiences.

Subsequent research has generally supported the dimensional model of adversity, but whether experiences involving harm and experiences involving threatened harm alone have similar effects has not yet been directly tested (McLaughlin et al., 2019). While the two types of experiences share many attributes (e.g., they may both induce fear), they may differ in important qualitative ways, and related evidence from both animal models and humans suggests they may lead to somewhat different effects. For example, some rodent models of traumatic stress use foot shock paradigms (possibly mimicking aspects of physically harmful experiences), while others expose rodents to a predator's scent (possibly mimicking experiences of threatened harm alone) (Lezak et al., 2017; Schöner et al., 2017). These paradigms elicit somewhat different biologic responses in rodents, suggesting that while both of them entail physically threatening experiences, they may impact brain function differently.

In humans, neural responses to fear-inducing stimuli partially depend on whether the stimuli cause pain (Biggs et al., 2020). Some neural correlates of pain-inducing and non-pain-inducing stimuli overlap, with the former being greater in magnitude than the latter (i.e., quantitative but not qualitative differences). This suggests neural responses are partly a function of stimulus intensity. However, in other regions of the brain, the two types of stimuli (pain-inducing and non-pain-inducing) may evoke opposing responses, which implies pain-dependent qualitative differences in neural responses independent from those due to stimulus intensity. For example, in the parieto-occipital sulcus, pain-inducing stimuli appear to evoke a positive response, while non-pain-inducing stimuli may evoke a negative response (Biggs et al., 2020). Thus, some short-term neural responses to pain versus the threat of pain may differ. By extension, it is possible that some longer-term responses to "harm" versus the threat of "harm" may also differ.

Other taxonomies of adverse experiences that are based on their presumed effects distinguish between instances of harm versus threat of harm. For example, since at least the 1700s, legal systems (specifically, the common law of intentional torts) have distinguished between threatening experiences where the perpetrator actually strikes the victim (i.e., "battery," hereafter referred to as "physical attack"), and those where the perpetrator threatens but does not actually strike the victim (i.e., "assault," hereafter referred to as "threatened violence") (William Blackstone, 1765). While this legal distinction developed without evidence from modern neuroscience technologies, it is nevertheless premised on defining types of experiences based on their specific consequences for victims, and it developed over centuries of observation.

Experiences of physical attack and threatened violence are common in the United States, though estimates of prevalence range widely depending on how researchers define violence exposure. Finkelhor et al. (2015) report that prevalence of "any physical assault" (a broad definition that aggregates physical attack, threatened violence, and other types of violence) among American youth aged 0 to 17 years exceeds 50% (Fin-

kelhor et al., 2015). Meanwhile, Kessler et al. (1995) report that 11% of men and 7% of women in the United States experience traumatic physical attack at some point in their lives (Kessler, 1995). Nevertheless, whether these distinct experiences may have similar or different neurodevelopmental consequences has not yet been tested. Our study aims to explore this knowledge gap.

Prior research has generally found that violence exposure (regardless of precise definition) is associated with smaller volumes in both gray matter, particularly in corticolimbic regions, and white matter, particularly in the corpus callosum, but these results have been somewhat inconsistent (Islam & Kaffman, 2021; McLaughlin et al., 2019; Teicher et al., 2016). The corpus callosum is the brain's largest white matter bundle, and it is involved in managing emotional and social responses among many other tasks (Islam & Kaffman, 2021). Separately, the brain's corticolimbic system, including the amygdala, hippocampus, anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC), is involved in threat perception and response (Holz et al., 2020; McLaughlin et al., 2019; Teicher et al., 2016). Smaller volumes in both the corpus callosum and corticolimbic regions have been associated with a spectrum of mental disorders (Islam & Kaffman, 2021; Teicher et al., 2016). Many of these disorders first occur in adolescence, a sensitive period of neurodevelopment marked by exceedingly rapid neural reorganization (Fuhrmann et al., 2015; Solmi et al., 2021). In turn, studying whether and how adverse experiences impact brain structure immediately prior to this period (i.e., in preadolescence) may inform our understanding of why so many mental disorders begin in adolescence.

However, studying possible differences in neurostructural effects of physical attack versus threatened violence is difficult for several reasons. Many neuroimaging studies of childhood violent experiences rely on clinical samples where children have often experienced both types of violence. This inhibits their ability to detect differing effects of co-occurring experiences because they often do not include enough participants exposed to only one of the two experiences. Moreover, these studies are often limited by sample size, further reducing their ability to detect differences between the two types of experiences. To overcome these limitations, this population neuroscience study uses a large sample of children from the general population, some of whom experienced physical attack, threatened violence, both types of violence, and neither type of violence.

This study uses data from the Generation R Study. When children were about ten years old, researchers collected retrospective data from mothers on their child's lifetime experiences with physical attack and threatened violence, and the children completed an MRI brain scan (White et al., 2018). Because human behavior entails coordinated activity across many brain regions, we hypothesized that physical attack and threatened violence experiences would each be associated with global brain differences, namely, smaller (1) cortical gray matter volume, (2) white matter volume, and (3) subcortical gray matter volume. We further hypothesized that physical attack experience would be asso-

ciated with greater volumetric differences than threatened violence experience. Finally, we postulated that any global cortical or subcortical volume differences would be due, in part, to differences in corticolimbic brain regions, i.e., the amygdala, hippocampus, anterior cingulate cortex, and orbitofrontal cortex.

MATERIAL AND METHODS

Participants

This study uses data from the Generation R Study, a population-based birth cohort in Rotterdam, the Netherlands, seeking to identify social, environmental, and genetic factors affecting child development (Jaddoe et al., 2012). The Generation R Study enrolled 9,978 new mother-infant dyads living in Rotterdam between 2002 and 2006. After securing written informed consent and assent from participants and their parents when appropriate, researchers have collected data from children and their caregivers at multiple times through the present. All consent forms and study protocols were and are approved by the Medical Ethics Committee of the Erasmus University Medical Center.

When participating children reached preadolescence (mean age 10.1 years, range 8.6 to 12.0), study researchers interviewed each child's primary caregiver, 96% of whom were mothers, about whether their child had ever experienced physical attack or threatened violence (White et al., 2018). At the same study center visit, staff scanned children with magnetic resonance imaging (MRI) (White et al., 2018). Primary analyses in this study included children with usable MRI data (described below) and reliable violence experience data reported by mothers. Among these children, we excluded those whose mothers reported using cocaine or heroin while pregnant. When twins and triplets were enrolled, we excluded all but one randomly selected sibling to avoid challenges with correlated data. Our final analytic sample included 2,905 children. Appendix A.1 provides more sample selection details.

Measures

Violence Experience

This study uses information from two different instruments, each administered at a different timepoint in the participants' childhoods, regarding instances of physically threatening experiences. These instruments, which are described in detail below, include: (1) an in-person maternal interview about their child's experiences with physical attack and / or threatened violence, which we used to derive our primary exposure measure; and (2) a postal questionnaire about corporal punishment practices, which mothers completed when their children were 8.1 years old. The corporal punishment questionnaire, which

we used in secondary analyses, assessed disciplinary tactics used by parents that may have involved experiences qualitatively similar to those of physical attack. However, our hypotheses are not confined to parent-perpetrated violence—they relate to all violent experiences regardless of perpetrator—but we use the corporal punishment data in secondary analyses to contextualize our primary analyses based on maternal interview data.

Physical attack and threatened violence.

During an in-person study center visit when children were preadolescents, trained study staff interviewed mothers about their child's experiences with stressful life events. The interview adapted items from Kendler's Life Stress Interview and Brown and Harris's Life Event and Difficulty Schedule (Amone-P'Olak et al., 2009; Brown & Harris, 1978; White et al., 2018). In the interview, mothers reported if their child had experienced any of 24 stressful life events at any point in time during his or her childhood (yes, no), including physical attack or threatened violence. English translations of questions asked in Dutch are (1) "Has anyone ever used physical violence against your child, for example, beaten [him / her] up?" (i.e., "physical attack"); and (2) "Has anyone ever threatened to use physical violence against your child, such that it didn't happen but your child was scared?" (i.e., "threatened violence"). Interviewers were trained to clarify that these questions referred to distinct types of non-overlapping experiences by ensuring that a single discrete event in the child's life could not be characterized as both physical attack and threatened violence. However, if a child initially experienced an instance of threatened violence and then, later in time, an instance of physical attack, the child's mother could report exposure to both types of experiences. Importantly, interviewers were also trained to clarify that the questions were not meant to capture *de minimis* experiences of physical attack or threatened violence, e.g., rough play or playground skirmishes. Interviewers deemed responses from mothers unreliable if language barriers inhibited the mother's question comprehension. We excluded these participants (n = 66).

Corporal Punishment.

When children were aged 8.1 years, mothers answered via postal questionnaire two questions regarding how often either slapping or spanking "typically occurs in the home" on a 5-point frequency scale ranging from "never" to "always" (Essau et al., 2006; Shelton & Frick, 1996). We summed these answers to construct a continuous score ranging from 0 to 8 quantifying each participant's corporal punishment experience. Appendix A.2 provides further detail.

Brain Imaging

Generation R researchers have described magnetic resonance imaging protocols elsewhere (White et al., 2018). All scans were acquired on a 3 Tesla GE Discovery MR750w scanner (General Electric, Milwaukee, WI, USA) yielding 1 mm isotropic resolution. Study staff processed resulting images in FreeSurfer v6.0.0, which estimated both global volumes and volumes for corticolimbic regions of interest (ROIs) in mm^3 (Fischl, 2012). Study researchers visually inspected each reconstruction and excluded poor quality images. In our primary analyses, we assessed three global volumes: (1) total cortical gray matter (all cortical tissue between the pial and white matter surfaces); (2) total cerebral white matter (white matter tissue inside the white matter surface, excluding cerebellar white matter and the brainstem); and (3) total subcortical gray matter (sum of volumes for the thalamus, caudate, putamen, pallidum, hippocampus, amygdala, and ventral diencephalon). ROIs included the amygdala, hippocampus, rostral and caudal anterior cingulate cortex (ACC), and lateral and medial orbitofrontal cortex (OFC).

Covariates

Researchers retrieved birthdate and sex data from birth records. Parents self-reported the following: their national origin and ethnicity, which we used to categorize child ethnicity as European (excluding Turkish), Turkish, Moroccan, Surinamese, and Other Ethnicity; household income during pregnancy (< or ≥ €2200 / month); highest maternal or paternal completed education level at study enrollment (less than high school equivalent; high school or intermediate vocational training; advanced vocational training, bachelor's degree, or higher); maternal and paternal history of psychotic episodes (yes / no for each parent); maternal age at childbirth; maternal smoking during pregnancy (never, until pregnancy known, or through pregnancy); and parental prenatal psychopathology symptoms assessed using the 53-item Brief Symptom Inventory (BSI) (Derogatis & Melisaratos, 1983). We calculated continuous BSI sum scores for each parent.

We imputed missing covariate (but not exposure or outcome) data. The proportion of missing data for most covariates was low (< 2%), except for household income (22%), maternal psychopathology symptoms (23%), partner educational attainment (36%), and partner psychopathology symptoms (38%). We imputed these missing values using the rich auxiliary data collected by Generation R researchers throughout the participants' lives that were predictive of missing covariate data, e.g., other socioeconomic indicators for partner educational attainment and partner history of psychosis for partner psychopathology symptoms (Harel et al., 2018; Perkins et al., 2018). To ensure we sufficiently modeled uncertainty around the imputed values, we created 50 imputed datasets, and we combined resulting estimates using Rubin's Rules (Rubin, 1996). Appendix A.3 includes additional imputation model details. For use in sensitivity analyses, we also calculated inverse probability of attrition weights to account for differential attrition by

sociodemographic characteristics. We deemed lost to follow-up any participant enrolled at baseline but excluded from our analytic sample for any reason. Appendix A.4 includes additional details regarding how these weights were derived.

Statistical Analyses

We excluded participants with global or ROI volumes over four standard deviations from the measure's analysis sample mean because such values are either biologically implausible or so far from the sample means that they likely represent pathology or brain structure abnormality (n = 14 excluded). Because we did not hypothesize hemisphere-specific effects, we averaged hemisphere-specific ROI volumes and standardized all measures. We used t-tests to assess sociodemographic differences in exposures. We calculated correlation coefficients between actual and threatened violence exposure and scores for harsh parenting and corporal punishment exposure.

In primary analyses, we used ordinary least squares (OLS)-estimated linear regression models to assess whether physical attack and threatened violence experiences were associated with continuous measures of the three global outcomes. For each outcome, we fit minimally adjusted models adjusting for scan age, sex, and ethnicity, and fully adjusted models incorporating all remaining covariates listed above (hereafter referred to as Primary Models). We additionally adjusted models of subcortical volume for total intracranial volume (ICV) to estimate whether physical attack or threatened violence were associated with subcortical volume differences *over and above* any global effects. Within each type of threatening experience, we adjusted *p*-values and calculated *q*-values for multiple tests via the Benjamini-Hochberg procedure, a method that controls the false discovery rate (FDR) when assuming non-negative correlation among estimates (3 global brain volumes, 3 tests) (Benjamini & Hochberg, 1995; White et al., 2019).

We fit several fully adjusted OLS-estimated sensitivity models to assess whether our results were robust to different sample constructions, model specifications, and modeling strategy assumptions. First, we fit linear models using inverse probability of attrition weights to address possible selection bias from differential attrition by sociodemographic variables (Sensitivity Model 1). Second, we fit a model including covariates for both physical attack and threatened violence exposure simultaneously (Sensitivity Model 2). Third, we fit models in subsamples excluding participants reporting both primary exposures, e.g., in models assessing physical attack, we excluded participants exposed to threatened violence (Sensitivity Model 3). Next, we fit marginal models of both primary exposures using both (1) inverse probability of exposure weights (Marginal Model 1) and (2) standardization via the parametric G-formula (Marginal Model 2) (Hernán & Robins, 2020). These models attempt to estimate population average exposure effects—as opposed to Primary Model effect estimates that are conditional on covariates—and thus require a different set of assumptions. Appendices A.4 and A.5 detail

these models more thoroughly. Thereafter, we re-fit Primary Models using a subsample of participants exposed either to physical attack or to threatened violence, but not to both types of experiences (n = 405). By excluding participants who experienced neither or both types of violence, these "Direct Comparison" models attempt to compare brain volumes of children who experienced physical attack only versus threatened violence only. Finally, to gain additional context for our subcortical volume findings, we fit ICV-unadjusted models, which we report in the Appendix, and which explore associations before accounting for global differences in overall head size.

In secondary analyses, we sought to clarify whether corticolimbic ROIs were affected by our primary exposures in ways that were similar to our global measures. Using the same modeling strategy detailed above, we fit ROI-specific models using continuous outcomes. For subcortical ROIs (i.e., amygdala and hippocampal volume), we fit models both adjusted and unadjusted for ICV. For these secondary analyses, we adjusted *p*-values and calculated *q*-values assuming 6 tests (6 brain ROIs) within each type of threatening experience via the Benjamini-Hochberg procedure (Benjamini & Hochberg, 1995).

Finally, we conducted secondary analyses assessing both global and ROI-specific associations with continuous corporal punishment scores using fully adjusted OLS-estimated models. We also fit these models additionally adjusting for physical attack exposure to assess whether estimates of either of these experiences (corporal punishment or physical attack) changed when considering the other.

After modeling our data, we interpreted results consistent with the American Statistical Association's guidance to evaluate the strength of statistical evidence based on effect sizes and confidence intervals, effect directions, and continuous p-values (Wasserstein & Lazar, 2016). In doing so, we minimize our reliance on *p*-value cutoffs in null hypothesis significance testing, though we use the language of statistical significance as a heuristic to concisely communicate certain results.

RESULTS

Analytic sample characteristics

Our primary analytic sample differed from the baseline cohort by sociodemographic characteristics. Included versus excluded participants were more likely to have European ethnicity (70% vs. 58%), parents with post-secondary educations (61% vs. 44%), and older mothers (mean maternal age at birth 31.6 vs. 29.8 years).

Of 2,905 children in our analytic sample, 202 experienced physical attack (Table 1). Boys were more likely than girls to have been exposed (9.8% vs. 4.1%), as were children with lower versus higher educated parents (8.8% vs. 5.6%). Separately, 335 children

experienced threatened violence, with similar patterns of differential exposure across sociodemographic groups to those above (Table 1). 66 children experienced both physical attack and threatened violence. Experiencing physical attack was moderately correlated with experiencing threatened violence (r = 0.19). Neither physical attack nor threatened violence were correlated with corporal punishment (r = -0.02 and r = 0.02, respectively).

Table 1. Distribution of primary and secondary exposures by participant characteristics in the primary analytic sample.

	Total	Physical Attack	Threatened Violence	Corporal Punishment
	n (%)	*n (%)*	*n (%)*	$\bar{x}$ *(s)*
Total sample	2905 (100.0)	202 (7.0)	335 (11.5)	0.6 (1.0)
Sex				
Female	1472 (50.7)	61 (4.1)	122 (8.3)	0.5 (1.0)
Male	1433 (49.3)	141 (9.8)	213 (14.9)	0.7 (1.0)
National origin / ethnicity				
European (non-Turkish)	1985 (69.6)	123 (6.2)	218 (11.0)	0.5 (0.9)
Turkish	148 (5.2)	8 (5.4)	12 (8.1)	0.6 (1.0)
Moroccan	126 (4.4)	8 (6.3)	14 (11.1)	1.3 (1.4)
Surinamese	212 (7.4)	23 (10.8)	30 (14.2)	1.0 (1.1)
Other	382 (13.4)	32 (8.4)	56 (14.7)	1.0 (1.3)
Household education				
Less than high school	116 (4.3)	7 (6.0)	10 (8.6)	0.8 (1.0)
High school equivalent	946 (34.7)	87 (9.2)	142 (15.0)	0.8 (1.1)
More than high school	1666 (61.1)	93 (5.6)	162 (9.7)	0.5 (0.9)
Household income				
€2200 / month or less	1442 (49.6)	126 (8.7)	195 (13.5)	0.8 (1.1)
More than €2200 / month	1463 (50.4)	76 (5.2)	140 (9.6)	0.5 (0.9)

a. This table is based on observed values for each characteristic and does not account for missing data.
b. $\bar{x}$ *and s denote sample mean and standard deviation, respectively.*
c. Corporal punishment scores were assessed at mean child age 8 years and have a range from 0 to 8.

Global brain volumes, primary and sensitivity analyses

In fully adjusted models, physical attack experience was associated with smaller total cortical gray matter and total white matter volume (Table 2). As illustrated in Figure 1, these results were robust to sample construction, model specification, and modeling strategy in most sensitivity analyses, though estimates from models excluding participants reporting both actual and threatened violence exposure were attenuated (Figure 1, Sensitivity Model 3). For example, the Primary Model estimate of the association between physical attack and cortical gray matter volume was $\beta_{\text{physical attack/cortical volume}} = -0.14$

(95% CI: -0.26, -0.02; p = 0.03; q = 0.04). In sensitivity models, these estimates ranged from β = -0.10 (95% CI: -0.24, 0.05) in Sensitivity Model 3 to β = -0.16 (95% CI: -0.32, -0.01) in Marginal Model 1, which used IPWs for exposure. Notably, the interpretation of the former estimate is conditional on included model covariates, while the latter is interpreted as the population average association. Separately, physical attack experience was associated with subcortical volume only *before* ICV adjustment. See Appendix B.7 and E.2. After adjusting for ICV, this relationship was no longer statistically significant: $\beta_{\text{physical attack/subcortical volume (ICV adjusted)}}$ = -0.05 (95% CI: -0.14, 0.03). Because adjusting for ICV attenuated this relationship, we found no statistically significant evidence that physical attack was associated with lower total subcortical volume over and above possible global effects (Table 2, Figure 1).

Table 2. Associations between childhood physical attack exposure, threatened violence exposure, and standardized global brain volumes in preadolescence. n = 2,905.

	Minimally adjusted models			Fully adjusted models			
Physical Attack	**ß**	**95% CI**	***p***	**ß**	**95% CI**	***p***	***q***
Cortical Gray Matter	-0.18	(-0.31, -0.06)	< 0.01	-0.14	(-0.26, -0.02)	0.03	0.04
White Matter	-0.19	(-0.31, -0.06)	< 0.01	-0.16	(-0.28, -0.03)	0.01	0.04
Subcortical Gray Matter	-0.05	(-0.13, 0.03)	0.23	-0.05	(-0.14, 0.03)	0.22	0.22
	Minimally adjusted models			**Fully adjusted models**			
Threatened Violence	**ß**	**95% CI**	***p***	**ß**	**95% CI**	***p***	***q***
Cortical Gray Matter	-0.01	(-0.11, 0.09)	0.87	0.04	(-0.06, 0.13)	0.45	0.68
White Matter	0.01	(-0.09, 0.11)	0.89	0.04	(-0.06, 0.14)	0.44	0.68
Subcortical Gray Matter	0.01	(-0.06, 0.08)	0.84	0.00	(-0.06, 0.07)	0.91	0.91

a. Minimally adjusted models include covariates for child age, sex, and ethnicity.
b. Fully adjusted models include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking.
c. Models of subcortical gray matter are additionally adjusted for intracranial volume (ICV). Results from ICV-unadjusted models, which answer a somewhat different but related scientific question, appear in Appendix Table B.7.
d. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method. q-values in this context can be conceptualized as "FDR-corrected" p-values.
e. Physical attack associations with (1) cortical gray matter and (2) white matter remain statistically significant after adjusting for multiple comparisons. No other associations are statistically significant.

We also found no evidence that threatened violence exposure (versus no exposure) was associated with total cortical or white matter volume in primary and sensitivity analyses, e.g., $\beta_{\text{threatened violence/cortical volume}}$ = 0.04 (95% CI: -0.06, 0.13) (Table 2, Figure 1). Compared with estimates for physical attack, those of threatened violence were smaller in magnitude and almost uniformly opposite in direction, i.e., nearly all point estimates were positive. Standard errors were relatively large compared to magnitudes, and none

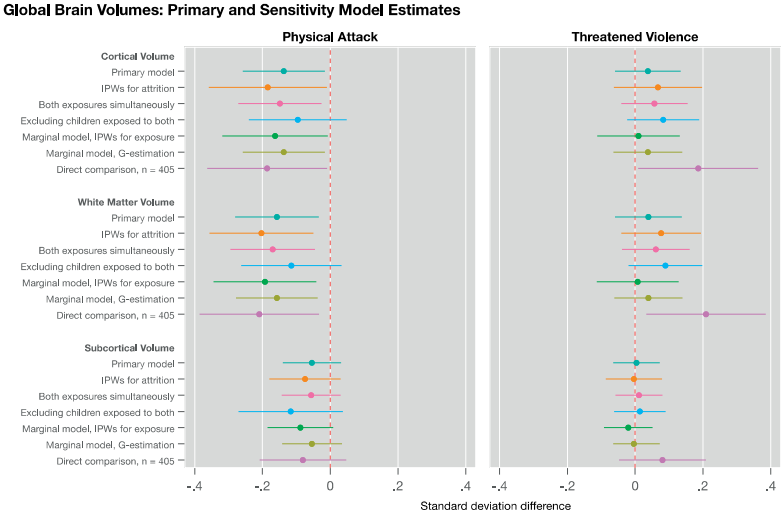

Figure 1. Associations between physical attack, threatened violence, and standardized global brain volumes using multiple modeling strategies. All models use sample size n = 2,905 unless otherwise stated.

Primary models are OLS-estimated linear regression models in the full analytic sample. n = 2,905.

Models using "IPWs for attrition" use inverse probability of attrition weights to account for selection bias (Sensitivity Model 1).

Models with "both exposures simultaneously" include covariates for both actual and mere threatened violence exposure simultaneously (Sensitivity Model 2).

Models "excluding children exposed to both" exclude participants exposed to both actual and mere threatened violence (Sensitivity Model 3). n = 2,570 for physical attack; n = 2,703 for threatened violence.

Marginal models using "IPWs for exposure" are fit using inverse probability of exposure weights.

Marginal models using G-Estimation are fit using standardization via the parametric G-formula.

Direct Comparison models use a subsample of participants exposed to either physical attack or threatened violence, but not to both of them. n = 405.

Estimates are from fully adjusted models accounting for child scan age, sex, ethnicity, household income, highest parental education level, maternal and paternal history of psychosis, maternal and paternal psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking. Models of subcortical volume are additionally adjusted for ICV.

Primary model estimates of the associations between (1) physical attack and cortical volume and (2) physical attack and white matter volume remain statistically significant after adjusting for multiple comparisons. See Table 1.

of these estimates were statistically significant at the $p = 0.05$ level. ICV-adjusted estimates of subcortical volume were close to zero with no consistent positive or negative pattern. Appendices B.1 through B.5 report sensitivity model results for global outcomes.

In Direct Comparison models, children who experienced physical attack only (versus threatened violence only) had smaller cortical ($\beta_{cortical}$ = -0.19; 95% CI: -0.36, -0.01) and white matter ($\beta_{white\ matter}$ = -0.21; 95% CI: -0.39, -0.03) volumes, and possibly smaller subcortical volumes after adjusting for ICV ($\beta_{Subcortical\ /\ ICV\ adjusted}$ = -0.08; 95% CI: -0.21, 0.05). See Figure 1, Figure 3, and Appendix B.6.

Corticolimbic brain volumes, primary and sensitivity analyses

Results from fully adjusted ROI analyses suggest physical attack exposure (versus no physical attack exposure) may be associated with smaller amygdala volume after ICV adjustment. In a Primary Model, $\beta_{\text{physical attack/amygdala (ICV adjusted)}}$ = -0.10 (95% CI: -0.21, 0.01) (Table 3, Figure 2), but this result was not statistically significant (p = 0.08, q = 0.24). Sensitivity model estimates were consistent and ranged from $\beta_{\text{physical attack/amygdala (ICV adjusted)}}$ = -0.13 (95% CI: -0.25, 0.00) in Marginal Model 1 (IPWs for exposure) to $\beta_{\text{physical attack/amygdala (ICV adjusted)}}$ = -0.10 (95% CI: -0.24, 0.05) in Sensitivity Model 1 (IPWs for attrition) (Appen-

Table 3. Associations between childhood physical attack exposure, threatened violence exposure, and standardized corticolimbic volumes in preadolescence. n = 2,905.

	Minimally adjusted models			Fully adjusted models			
Physical Attack	ß	95% CI	*p*	ß	95% CI	*p*	*q*
Amygdala Volume	-0.09	(-0.21, 0.02)	0.10	-0.10	(-0.21, 0.01)	0.08	0.24
Hippocampus Volume	-0.03	(-0.14, 0.09)	0.63	-0.03	(-0.14, 0.09)	0.64	0.72
Anterior Cingulate Cortex							
Rostral Volume	-0.10	(-0.23, 0.04)	0.16	-0.07	(-0.21, 0.06)	0.30	0.45
Caudal Volume	0.01	(-0.13, 0.15)	0.91	0.03	(-0.12, 0.17)	0.72	0.72
Orbitofrontal Cortex							
Medial Volume	-0.12	(-0.25, -0.01)	0.08	-0.09	(-0.22, 0.04)	0.17	0.34
Lateral Volume	-0.16	(-0.30, -0.03)	0.02	-0.13	(-0.26, 0.01)	0.06	0.24
	Minimally adjusted models			Fully adjusted models			
Threatened Violence	ß	95% CI	*p*	ß	95% CI	*p*	*q*
Amygdala Volume	0.04	(-0.05, 0.13)	0.43	0.03	(-0.06, 0.12)	0.56	0.62
Hippocampus Volume	0.06	(-0.03, 0.15)	0.16	0.06	(-0.03, 0.15)	0.18	0.54
Anterior Cingulate Cortex							
Rostral Volume	0.02	(-0.08, 0.13)	0.66	0.05	(-0.06, 0.16)	0.40	0.60
Caudal Volume	0.04	(-0.07, 0.15)	0.50	0.05	(-0.06, 0.17)	0.36	0.60
Orbitofrontal Cortex							
Medial Volume	0.07	(-0.03, 0.18)	0.16	0.10	(-0.00, 0.21)	0.06	0.36
Lateral Volume	-0.01	(-0.12, 0.10)	0.86	0.03	(-0.08, 0.13)	0.62	0.62

a. Minimally adjusted models include covariates for child age, sex, and ethnicity.

b. Fully adjusted models include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking.

c. Models of amygdala and hippocampus volume are additionally adjusted for intracranial volume (ICV). Results from ICV-unadjusted models, which answer a somewhat different but related scientific question, appear in Appendix Table C.7 and C.8.

d. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method. q-values in this context can be conceptualized as "FDR-corrected" p-values.

e. Of note, none of the fully adjusted estimates listed in this table are statistically significant at the p = 0.05 level.

dices C.1-C.5). Results also suggest a possible relationship between physical attack and smaller lateral OFC volume: $\beta_{\text{physical attack/lateral OFC}}$ = -0.13 (95% CI: -0.26, 0.01; p = 0.06; q = 0.24) from the Primary Model, while most sensitivity models yielded comparable results. Evidence of a similar relationship between physical attack and smaller medial OFC was comparatively weaker but nonetheless noteworthy in context, e.g., Primary Model $\beta_{\text{physical attack/medial OFC}}$ = -0.09 (95% CI: -0.22, 0.04; p = 0.17; q = 0.34). We found no other evidence of associations between physical attack and any other ROI.

Our results also provide weak evidence of a possible relationship between threatened violence exposure (versus no exposure) and larger medial OFC volume (Table 3, Figure 2). For example, in the Primary Model, $\beta_{\text{threatened violence/medial OFC}}$ = 0.10 (95% CI: -0.00,

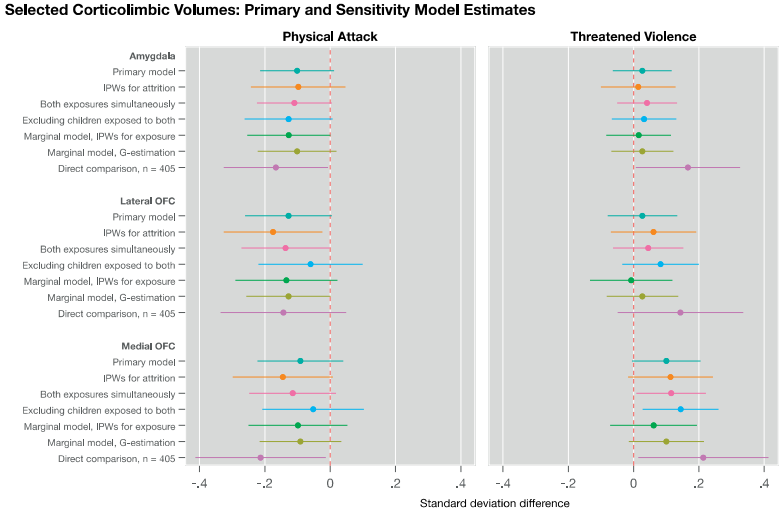

Figure 2. Associations between physical attack, threatened violence, and selected standardized corticolimbic volumes using multiple modeling strategies. All models use sample size n = 2,905 unless otherwise stated.

Primary models are OLS-estimated linear regression models in the full analytic sample. n = 2,905.

Models using "IPWs for attrition" use inverse probability of attrition weights to account for selection bias (Sensitivity Model 1).

Models with "both exposures simultaneously" include covariates for both actual and mere threatened violence exposure simultaneously (Sensitivity Model 2).

Models "excluding children exposed to both" exclude participants exposed to both actual and mere threatened violence (Sensitivity Model 3). n = 2,570 for physical attack; n = 2,703 for threatened violence.

Marginal models using "IPWs for exposure" are fit using inverse probability of exposure weights.

Marginal models using G-Estimation are fit using standardization via the parametric G-formula.

Direct Comparison models use a subsample of participants exposed to either physical attack or threatened violence, but not to both of them. n = 405.

Estimates are from fully adjusted models accounting for child scan age, sex, ethnicity, household income at birth, highest parental education level achieved, maternal and paternal history of psychosis, maternal and paternal psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking. Models of amygdala volume are additionally adjusted for ICV.

Notably, none of the fully adjusted primary model estimates above are statistically at the p = 0.05 level before or after adjusting for multiple comparisons.

0.21; $p = 0.06$; $q = 0.36$), and in Sensitivity Model 2 (modeling both exposures simultaneously), $\beta_{\text{threatened violence/medial OFC}} = 0.12$ (95% CI: 0.01, 0.22; $p = 0.03$; $q = 0.18$). While we found no evidence of associations between threatened violence and any other corticolimbic ROI, results from all such models evinced a pattern in which nearly every estimate was positive (see, e.g., Figure 2). Appendices C.1 through C.5 report ROI sensitivity model results.

In Direct Comparison models, physical attack exposure (versus threatened violence exposure) was also associated with smaller volumes in the amygdala (both ICV-unadjusted and -adjusted) and medial OFC, with weaker evidence of similar differences in hippocampal and lateral OFC volumes. (Figure 2, Figure 3, Appendix C.6, Appendix E.1). These models revealed no evidence of volume differences in either ACC region.

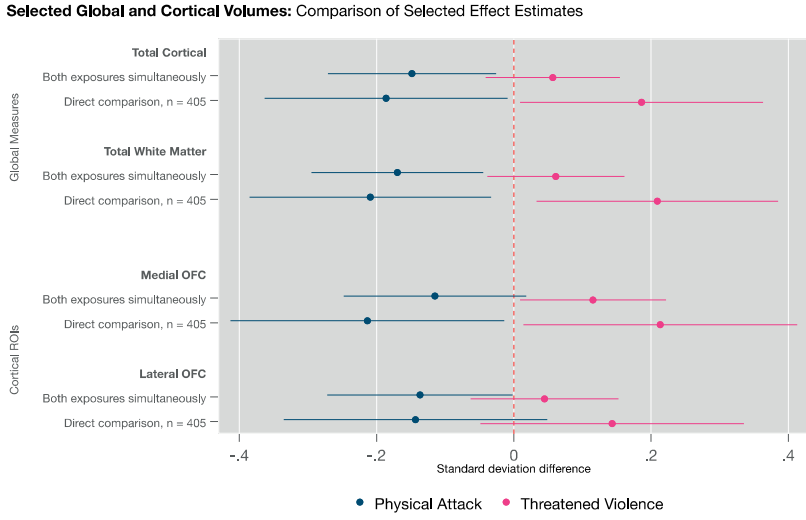

Figure 3. Associations between physical attack (navy), threatened violence (pink), and selected standardized brain volumes in selected sensitivity models.
Models with "both exposures simultaneously" include covariates for both actual and mere threatened violence exposure simultaneously (Sensitivity Model 2). n = 2,905.
Direct Comparison models use a subsample of participants exposed to either physical attack or threatened violence, but not to both of them. n = 405. Because all participants in this subsample experienced one or the other type of violence, effect estimates mirror each other.
Estimates are from fully adjusted models accounting for child scan age, sex, ethnicity, household income at birth, highest parental education level achieved, maternal and paternal history of psychosis, maternal and paternal psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.

Secondary analyses

In secondary analyses, a higher corporal punishment score was associated with smaller global (total cortical and white matter) and cortical ROI volumes (rostral and caudal ACC, medial and lateral OFC), but not subcortical ROI volumes (amygdala, hippocampus) after ICV adjustment (Appendix D.1). Adding a covariate for physical attack to models

of corporal punishment did not markedly change the corporal punishment estimate for any outcome (Appendix D.2). Similarly, estimates for physical attack were mostly similar with and without additionally adjusting for corporal punishment. The exception was for amygdala volume, e.g., before adjusting for corporal punishment score, $\beta_{\text{physical attack/amygdala (ICV adjusted)}} = -0.10$ (95% CI: -0.21, 0.01); but afterward, $\beta_{\text{physical attack/amygdala (ICV adjusted)}} = -0.20$ (95% CI: -0.34, -0.05).

DISCUSSION

This study explored and compared associations between two types of physically threatening experiences—physical attack and threatened violence—and preadolescent brain structure. Despite similarities between these experiences (e.g., both may induce fear), our results suggest physical attack and threatened violence may have quantitatively different effects on both global and corticolimbic brain structure.

Specifically, physical attack experience was associated with smaller total cortical and white matter volume. Follow-up corticolimbic ROI analyses suggested that physical attack may also be associated with smaller amygdala, lateral OFC, and possibly medial OFC volumes, though these results were not statistically significant. Consistent estimates of these associations across multiple modeling strategies decreases the likelihood that the results are spurious due to model misspecification or sample construction.

Our measure of physical attack captured a spectrum of experiences—from aggressive fighting to parental physical abuse—while our corporal punishment measure captured a narrower range of parent-perpetrated experiences. Nevertheless, analyses of corporal punishment experience enable a form of replication of our physical attack findings because both experiences entail instances of children being physically struck without their consent, e.g., being spanked, slapped, or beaten up. Thus, results from both measures (physical attack and corporal punishment)—each assessed at a different time and capturing a slightly different set of physically violent experiences—converge on a central finding: on average, physical attack experience in childhood is associated with smaller global and possibly some corticolimbic brain volumes in preadolescence in a population-based sample.

In contrast, we found no evidence of comparable associations between threatened violence experience and smaller brain volumes similar to those of physical attack. None of the threatened violence effect estimates for either global or ROI outcomes were statistically significant after FDR adjustment. Moreover, the direction of nearly all such estimates—though small in magnitude, highly uncertain, and statistically non-significant—was positive, i.e., the estimates were in the opposite direction compared to those of physical attack. Direct Comparison models provide further evidence that

effects of threatened violence differ from those of physical attack, at least in magnitude. Compared directly to children who experienced only threatened violence, children who experienced only physical attack had smaller volumes in most global and corticolimbic outcomes. Thus, results from Direct Comparison models suggest quantitative differences in effects between physical attack and threatened violence.

These results are consistent with a number of possible scenarios. The first scenario is that while experiences of physical attack have a negative effect on some preadolescent brain volumes, those of threatened violence (as they are measured and operationalized in this study) have no enduring effect on brain volumes. A second possible scenario is that experiences of threatened violence have small negative effects on brain volumes—akin to those of physical attack but smaller in magnitude, which is what we originally hypothesized—and our study was simply unable to detect them. Perhaps our large, population-based sample was nevertheless statistically underpowered, or our measures were too imprecise. Under this second scenario, differences in effect magnitude between the two types of experiences may be due to exposure severity. Both physical attack and threatened violence may affect the same regions of the brain in similar ways, with the latter being a less impactful manifestation of the former. However, if the two types of experiences differed only by severity, we might expect that at least some effect estimates for both experiences would have shared directionality (if not magnitude), but they did not, though substantial uncertainty surrounded many of them. In any event, whether the first scenario (threatened violence has no effects) or the second scenario (threatened violence has negative effects but we did not detect them) is correct, our results suggest quantitative differences in effects between experiences of physical attack and threatened violence.

There is also a third—albeit less likely—scenario that may warrant further investigation in future research. Namely, the near-uniform pattern in which effect estimates for physical attack versus threatened violence are in opposite directions hints at possible *qualitative* differences in effects. Under this scenario, physical attack may lead to some smaller brain volumes, while threatened violence may lead to some larger volumes. Differences in effect direction (i.e., qualitative differences) could be due to allostatic processes. Models of allostasis, i.e., stress-adaptive biologic processes that interact in nonlinear ways to maintain homeostasis, posit differing neuronal effects depending on stressor severity and chronicity (Hanson & Nacewicz, 2021; McEwen et al., 2015). Less acute stress may increase neuronal stimulation and excitation, which may manifest structurally as volumetric increases, while more acute or longer-lasting stress may lead to cell death and volumetric decreases. These effects may also be heterogeneous across brain regions (McEwen et al., 2015). Notably, while possible qualitative differences in neural effects of physical attack and threatened violence are not easily explained by existing models of adversity, similar differences may not be without precedent: as re-

viewed above, some neural correlates of fear-inducing stimuli appear to depend on the presence or absence of pain (Biggs et al., 2020). Nevertheless, while this scenario may warrant additional investigation, it remains an unlikely possibility. Threatened violence effect sizes were exceedingly small for all outcomes, none were statistically significant after FDR correction, and all of them were based on responses to a single interview question posed to mothers. Moreover, none of this evidence should be construed to suggest that experiences of threatened violence confer "positive" effects on children.

Our study reflects some aspects of specificity models of childhood adversity because it independently tested effects of qualitatively different experiences. However, our study was also informed by the dimensional model of adversity, and our findings bear on aspects of it in two ways. First, the dimensional model argues that effects of adversity scale based on experience frequency and severity. In practice, studies exploring this aspect of the dimensional model (at least as it relates to threat) have created threat "severity scores" by summing the discrete types threatening experiences to which a child has been exposed (McLaughlin et al., 2016; Weissman et al., 2020). Implicit in this practice is that different types of threatening experiences will have additive effects, much the same way cumulative risk models sum exposures to all types of adversity. Our findings suggest that the effect magnitude of some threats may be different than that of others, such that creating severity scores in this way may not accurately reflect the underlying severity of a child's overall exposure. Second, in contrast to the dimensional model, our study hints at the possibility that experiences of physical attack and threatened violence may have some qualitatively different effects. Additional research in population-based samples large enough to isolate effects of specific types of threatening experiences on specific brain regions may clarify this question.

Identifying possible differences in neurodevelopmental effects of physical attack and threatened violence also has public health significance. Gaining a greater understanding of the neural mechanisms mediating relationships between specific types of violence exposure and child mental wellbeing can clarify how the brain changes in response to specific types of adversity. Ultimately, this type of research may help provide insight into understanding what types of interventions may enable children facing adversity to reach their full potential. Moreover, explanatory models of childhood adversity—including the dimensional model of childhood adversity—can be exceedingly useful in guiding policy and mobilizing public health resources, but only if they are premised on scientifically sound assumptions. It is therefore important to test these assumptions to ensure the model's translational impact.

Our study has some limitations. Because data for our primary exposures and outcomes were collected at the same time, our study is cross-sectional. We used retrospective maternal reports of violent experiences because Generation R did not collect child-report data on them. Mothers may not have known about, remembered, or wanted to

report all instances of the two types of experiences. They also may have been less likely to know about or recall threatened violence experiences than physical attack experiences because instances of the latter may have led to injury or seemed more impactful. Mothers also may not have viewed corporal punishment as physical attack, particularly because "physical attack" was defined in Generation R as "beat[ing] up" the child. This may explain why corporal punishment scores were not correlated with physical attack. We partially addressed some these concerns by testing corporal punishment exposure separately, which was assessed prospectively at a different age. Neither our hypotheses nor our models account for experience timing, i.e., the age when children were exposed. Emerging research suggests timing of adversity exposure may impact the effects of it (Dunn et al., 2019; Gabard-Durnam & McLaughlin, 2019; Nelson & Gabard-Durnam, 2020). Our models also do not account for experience frequency or severity; thus, we are unable to test directly whether effects scale based on frequency and severity. Our study does not account for possible differences in pubertal status of our participants, though we included both age at MRI scan and sex as covariates, which may partially account for these differences. Differential attrition in the cohort by sociodemographic characteristics limits the study's generalizability, but our use of inverse probability of attrition weights reduces concerns about selection bias. Finally, as with all observational studies, confounding and reverse causation may have biased our results.

Our study also has significant strengths. Trained Generation R researchers collected our primary exposure data via in-person maternal interviews, which enabled researchers to clarify mothers' questions about what specific types of experiences constituted physical attack versus threatened violence. Similarly, our sample was large enough to investigate two frequently co-occurring experiences and to isolate their possible effects. Our sample was also more likely to capture less severe forms of these experiences than samples in which violence-exposed children are specifically recruited. Moreover, we were able to partially replicate findings using an independent measure (corporal punishment), which was assessed at a different timepoint in the participants' lives. Finally, we employed a variety of modeling strategies to assess the robustness of our results.

Conclusions

In our population-based sample of 2,905 children, experiences of physical attack—but not of threatened violence—were associated with smaller preadolescent global brain and some corticolimbic volumes. These results suggest that two types of threatening experiences may have quantitatively—and perhaps qualitatively—different neurodevelopmental consequences. Future studies in population-based samples large enough to isolate effects of frequently co-occurring experiences may confirm or refine aspects of dimensional models of adversity.

More broadly, our study contributes to research exploring how threatening experiences may affect brain development, which has important public health consequences. Prior studies suggest differences in corticolimbic function mediate associations between violent experiences and child mental disorders and behavior problems, while our findings suggest different types of violence exposure may have different effects on corticolimbic phenotype (McLaughlin & Lambert, 2017). In turn, our study provides additional context when untangling the complex neurodevelopmental and behavioral response to childhood violence exposure and adversity.

DECLARATION OF INTERESTS

The authors report no conflicts of interest related to this research.

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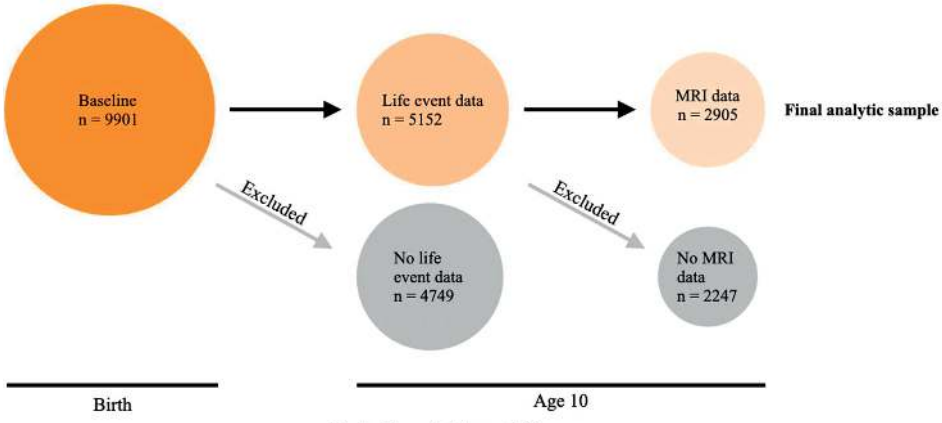
APPENDICES OF SUPPLEMENTAL INFORMATION

A. Measures and Methods

A.1. Sample composition.



Missing life event data, n = 4749

4314	No life event interview
292	Interviewee not mother
66	Interview answers deemed unreliable by interviewer
18	In utero exposure to heroin or cocaine
59	Randomly selected twin removed

Missing MRI data, n = 2247

1542	No MRI consent
24	Incidental MRI finding
660	Unusable image reconstruction
21	Outlier +/- 4 SD from the mean

Demographic characteristics after differential attrition.

	Life event data		Life event + MRI data	
	Included	Excluded	Included	Excluded
n	5152	4535	2905	6996
Female	51%	48%	51%	49%
European	69%	52%	70%	58%
High parent income	49%	25%	50%	32%
High parent education	55%	32%	61%	38%

**Percentages based on observed values and do not account for missing data.*

A.2. Corporal punishment.

When participating children were 8.1 years old (range 7.5 - 10.0), 4,654 mothers completed a postal questionnaire containing 41 items from the Alabama Parenting Questionnaire (APQ). The APQ measures how often both positive and negative parenting practices "typically occur in the home" on a 5-point frequency scale ranging from "Never" to "Always".[1,2] It includes a corporal punishment subscale of three items, though

Generation R study staff excluded one item due to Institutional Review Board considerations because it asked about instances of child abuse. The remaining two items of the subscale asked how often mothers either slapped or spanked their children when they did something wrong. We constructed a continuous sum score using both items resulting in a possible range from 0 to 8.

A.3. Multiple imputation models.

We imputed missing covariate data. We used the 'mi impute chained' function in Stata 16.1/MP to conduct multiple imputation by chained equations. We specified linear regression models for continuous variables and used predictive mean matching for all other variables (knn = 10). We specified a burn-in period of 20 iterations to ensure convergence to a stationary posterior distribution. We created 50 imputed datasets and combined resulting estimates using Rubin's Rules.[4]

A.4. Inverse probability of attrition weights

We defined participants lost to follow up as those enrolled at baseline but excluded from our analysis sample for any reason. To calculate our IPWs, we identified a broad set of variables theorized to predict who among originally enrolled participants satisfied our inclusion criteria. We used the 'mi impute chained' package in Stata 16.1/MP to conduct multiple imputation by chained equations (linear regression for continuous variables; predictive mean matching for all other variables, knn = 10; burn-in = 25) to address missing data in these variables, resulting in 100 imputed datasets. Next, we used Rubin's Rules to collapse resulting estimates.[3] Thereafter, we fit logistic regression models using these variables to predict the likelihood of each enrolled participant's inclusion in our analysis sample. Finally, we calculated IPWs for use in later analyses. Unstabilized weights had a mean of 0.95 and ranged from 0.40 to 22.80.

A.5. Construction of marginal models using inverse probability weights.

We used logistic regression to model the propensity of each exposure (i.e., physical attack exposure and threatened violence exposure) using all covariates from our fully adjusted models, then calculated the inverse of the predicted exposure propensity for each participant and used the resulting weights in marginal OLS-estimated linear regression models consisting only of the respective exposure and outcome.[4] Stabilized weights for models of physical attack exposure had mean 1.00 and range 0.30 to 2.45. For threatened violence exposure, stabilized weights had mean 1.00 and range 0.30 to 2.24.

A.6. Construction of marginal models using standardization via the parametric G-formula.

For each exposure-outcome combination, we fit a fully adjusted ordinary least squares linear regression model including the same covariates used elsewhere in this study. Next, we used the resulting parameter estimates to predict outcome values for two hypothetical datasets: the first assuming no participants were exposed to the exposure, and the second assuming all participants were exposed. Finally, we subtracted the mean predicted outcome value from the former hypothetical dataset (assuming no one had been exposed) from the mean predicted outcome value from the latter hypothetical dataset (assuming everyone had been exposed) to obtain a standardized mean estimate of the association between each exposure-outcome combination.[4] We calculated standard errors and 95% confidence intervals using the bootstrap method with 1,000 bootstrap samples within each imputation and combined resulting estimates using Rubin's Rules.[3]

B. Additional Results for Global Brain Volumes

Appendix Table B.1: Associations between childhood physical attack, threatened violence exposure, and standardized global brain volumes in preadolescence in models weighted to account for differential attrition by sociodemographic characteristics. n = 2,905. (Sensitivity Model 1)

	Physical Attack				Threatened Violence			
	β	95% CI	*p*	*q*	β	95% CI	*p*	*q*
Cortical Brain Volume	-0.18	(-0.36, -0.01)	0.04	0.06	0.07	(-0.06, 0.20)	0.31	0.47
White Matter Volume	-0.20	(-0.36, -0.05)	0.01	0.03	0.08	(-0.04, 0.19)	0.20	0.47
Subcortical Brain Volume	-0.07	(-0.18, 0.03)	0.17	0.17	0.00	(-0.09, 0.08)	0.93	0.93

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.

b. All models use inverse probability of attrition weights to account for possible selection bias due to differential attrition from baseline by sociodemographic characteristics.

c. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

Appendix Table B.2: Associations between actual violence exposure, threatened violence exposure, and standardized global brain volumes in preadolescence in models including both exposure variables simultaneously. n = 2,905. (Sensitivity Model 2)

	Physical Attack				Threatened Violence			
	Β	95% CI	*p*	*q*	Β	95% CI	*p*	*q*
Cortical Brain Volume	-0.15	(-0.27, -0.03)	0.02	0.03	0.06	(-0.04, 0.15)	0.26	0.39
White Matter Volume	-0.17	(-0.29, -0.04)	< 0.01	0.01	0.06	(-0.04, 0.16)	0.23	0.39
Subcortical Brain Volume	-0.06	(-0.14, 0.03)	0.21	0.21	0.01	(-0.06, 0.08)	0.75	0.75

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

Appendix Table B.3: Associations between physical attack, threatened violence exposure, and standardized global brain volumes in preadolescence after excluding participants reporting both types of experiences. (Sensitivity Model 3)

	Physical Attack, n = 2,570				Threatened Violence, n = 2,703			
	Β	95% CI	*p*	*q*	Β	95% CI	*p*	*q*
Cortical Brain Volume	-0.10	(-0.24, 0.05)	0.19	0.29	0.08	(-0.02, 0.19)	0.13	0.19
White Matter Volume	-0.11	(-0.26, 0.03)	0.13	0.29	0.09	(-0.02, 0.20)	0.11	0.19
Subcortical Brain Volume	-0.05	(-0.15, 0.06)	0.37	0.37	0.01	(-0.06, 0.09)	0.72	0.72

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

Appendix Table B.4: Marginal models of associations between childhood physical attack, threatened violence exposure, and standardized global brain volumes in preadolescence; marginal models constructed using inverse probability of exposure weights. n = 2,905. (Marginal Model 1)

	Physical Attack				Threatened Violence			
	Β	95% CI	*p*	*q*	Β	95% CI	*p*	*q*
Cortical Brain Volume	-0.16	(-0.32, -0.01)	0.04	0.06	0.01	(-0.11, 0.13)	0.87	0.90
White Matter Volume	-0.19	(-0.34, -0.04)	0.01	0.03	0.01	(-0.11, 0.13)	0.90	0.90
Subcortical Brain Volume	-0.09	(-0.18, 0.01)	0.08	0.08	-0.02	(-0.09, 0.05)	0.58	0.90

a. Exposure probability models were fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

Appendix Table B.5: Marginal models of associations between childhood physical attack, threatened violence exposure, and standardized global brain volumes in preadolescence using standardization via the parametric g-formula. n = 2,905. (Marginal Model 2)

	Physical Attack				Threatened Violence			
	Ƀ	95% CI	*p**	*q*	Ƀ	95% CI	*p**	*q*
Cortical Brain Volume	-0.14	(-0.26, -0.02)	0.03	0.05	0.04	(-0.06, 0.14)	0.48	0.72
White Matter Volume	-0.16	(-0.28, -0.04)	0.01	0.03	0.04	(-0.06, 0.14)	0.45	0.72
Subcortical Brain Volume	-0.05	(-0.14, 0.03)	0.23	0.23	0.00	(-0.06, 0.07)	0.92	0.92

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.

b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

** P-values calculated after estimation based on bootstrap confidence intervals.*

Appendix Table B.6: Associations between childhood physical attack versus threatened violence exposure and standardized global brain volumes in preadolescence in models including only participants exposed to either physical attack or threatened violence, but not to both of them. n = 405. (Direct Comparison Model)

	Physical Attack				Threatened Violence			
Global Measures	Ƀ	95% CI	*p*	*q*	Ƀ	95% CI	*p*	*q*
Cortical Gray Matter	-0.19	(-0.36, -0.01)	0.04	0.06	0.19	(0.01, 0.36)	0.04	0.06
White Matter	-0.21	(-0.39, -0.03)	0.02	0.06	0.21	(0.03, 0.39)	0.02	0.06
Subcortical Gray Matter	-0.08	(-0.21, 0.05)	0.22	0.22	0.08	(-0.05, 0.21)	0.22	0.22

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.

b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

c. Because all participants in this subsample experienced one or the other type of violence, effect estimates mirror each other.

Appendix Table B.7. Associations between childhood physical attack exposure, threatened violence exposure, and total subcortical volume with and without adjusting for ICV, primary and sensitivity models. n = 2,905.

	Physical Attack			Threatened Violence		
	Β	95% CI	*p*	Β	95% CI	*p*
Primary Model						
ICV unadjusted	-0.15	(-0.28, -0.02)	0.02	0.02	(-0.08, 0.12)	0.73
ICV adjusted	-0.05	(-0.14, 0.03)	0.22	0.00	(-0.06, 0.07)	0.91
Sensitivity Model 1						
ICV unadjusted	-0.21	(-0.38, -0.04)	0.02	0.04	(-0.08, 0.16)	0.47
ICV adjusted	-0.07	(-0.18, 0.03)	0.17	0.00	(-0.09, 0.08)	0.93
Sensitivity Model 2						
ICV unadjusted	-0.16	(-0.29, -0.03)	0.02	0.04	(-0.07, 0.14)	0.47
ICV adjusted	-0.06	(-0.14, 0.03)	0.21	0.01	(-0.06, 0.08)	0.75
Sensitivity Model 3						
ICV unadjusted	-0.12	(-0.27, 0.04)	0.14	0.06	(-0.06, 0.17)	0.33
ICV adjusted	-0.05	(-0.15, 0.06)	0.37	0.01	(-0.06, 0.09)	0.72
Marginal Model 1						
ICV unadjusted	-0.21	(-0.37, -0.05)	0.01	-0.04	(-0.16, 0.09)	0.57
ICV adjusted	-0.09	(-0.18, 0.01)	0.08	-0.02	(-0.09, 0.05)	0.58
Marginal Model 2						
ICV unadjusted	-0.15	(-0.28, -0.02)	0.02	0.02	(-0.08, 0.12)	0.75
ICV adjusted	-0.05	(-0.14, 0.03)	0.23	0.00	(-0.06, 0.07)	0.92
Direct Comparison Model						
ICV unadjusted	-0.19	(-0.38, -0.01)	0.04	0.19	(0.01, 0.38)	0.04
ICV adjusted	-0.08	(-0.21, 0.05)	0.22	0.08	(-0.05, 0.21)	0.22

All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking.

Section C. Additional Results for Corticolimbic Brain Volumes

Appendix Table C.1: Associations between childhood physical attack, threatened violence exposure, and standardized corticolimbic brain volumes in preadolescence in models weighted to account for differential attrition by sociodemographic characteristics. n = 2,905. (Sensitivity Model 1)

	Physical Attack				Threatened Violence			
	Β	95% CI	*p*	*q*	Β	95% CI	*p*	*q*
Amygdala Volume	-0.10	(-0.24, 0.05)	0.19	0.38	0.01	(-0.10, 0.13)	0.81	0.81
Hippocampus Volume	-0.05	(-0.19, 0.10)	0.54	0.65	0.05	(-0.06, 0.15)	0.39	0.48
Anterior Cingulate Cortex								
Rostral Volume	-0.09	(-0.26, 0.08)	0.32	0.48	0.06	(-0.08, 0.19)	0.40	0.48
Caudal Volume	0.00	(-0.19, 0.18)	0.98	0.98	0.09	(-0.06, 0.23)	0.24	0.48
Orbitofrontal Cortex								
Medial Volume	-0.15	(-0.30, 0.01)	0.06	0.18	0.11	(-0.02, 0.24)	0.09	0.48
Lateral Volume	-0.18	(-0.33, -0.02)	0.02	0.12	0.06	(-0.07, 0.19)	0.36	0.48

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. All models use inverse probability of attrition weights to account for possible selection bias due to differential attrition from baseline by sociodemographic characteristics.
c. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

Appendix Table C.2: Associations between actual violence exposure, threatened violence exposure, and standardized corticolimbic brain volumes in preadolescence in models including both exposure variables simultaneously. n = 2,905. (Sensitivity Model 2)

	Physical Attack				Threatened Violence			
	Β	95% CI	*p*	*q*	Β	95% CI	*p*	*q*
Amygdala Volume	-0.11	(-0.22, 0.00)	0.06	0.18	0.04	(-0.05, 0.13)	0.38	0.42
Hippocampus Volume	-0.04	(-0.16, 0.07)	0.48	0.58	0.07	(-0.02, 0.16)	0.15	0.42
Anterior Cingulate Cortex								
Rostral Volume	-0.09	(-0.22, 0.05)	0.23	0.35	0.06	(-0.05, 0.17)	0.30	0.42
Caudal Volume	0.02	(-0.13, 0.16)	0.83	0.83	0.05	(-0.06, 0.17)	0.38	0.42
Orbitofrontal Cortex								
Medial Volume	-0.11	(-0.25, 0.02)	0.09	0.18	0.12	(0.01, 0.22)	0.03	0.18
Lateral Volume	-0.14	(-0.27, -0.00)	0.05	0.18	0.04	(-0.06, 0.15)	0.42	0.42

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

Appendix Table C.3: Associations between physical attack, threatened violence exposure, and standardized corticolimbic brain volumes in preadolescence after excluding participants reporting both types of experiences. (Sensitivity Model 3)

	Physical Attack, n = 2,570				Threatened Violence, n = 2,703			
	Β	95% CI	*p*	*q*	Β	95% CI	*p*	*q*
Amygdala Volume	-0.13	(-0.26, 0.01)	0.06	0.36	0.03	(-0.07, 0.13)	0.53	0.53
Hippocampus Volume	-0.02	(-0.16, 0.11)	0.74	0.74	0.08	(-0.02, 0.18)	0.12	0.26
Anterior Cingulate Cortex								
Rostral Volume	-0.03	(-0.19, 0.14)	0.74	0.74	0.09	(-0.03, 0.21)	0.16	0.26
Caudal Volume	0.04	(-0.13, 0.21)	0.68	0.74	0.06	(-0.07, 0.19)	0.35	0.42
Orbitofrontal Cortex								
Medial Volume	-0.05	(-0.21, 0.10)	0.51	0.74	0.14	(0.03, 0.26)	0.02	0.12
Lateral Volume	-0.06	(-0.22, 0.10)	0.46	0.74	0.08	(-0.03, 0.20)	0.17	0.26

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

Appendix Table C.4: Marginal models of associations between childhood physical attack, threatened violence exposure, and standardized corticolimbic brain volumes in preadolescence; marginal models constructed using inverse probability of exposure weights. n = 2,905. (Marginal Model 1)

	Physical Attack				Threatened Violence			
	Β	95% CI	*p*	*q*	Β	95% CI	*p*	*q*
Amygdala Volume	-0.13	(-0.25, 0.00)	0.05	0.27	0.02	(-0.08, 0.11)	0.76	0.91
Hippocampus Volume	-0.06	(-0.19, 0.06)	0.32	0.38	0.06	(-0.03, 0.16)	0.17	0.72
Anterior Cingulate Cortex								
Rostral Volume	-0.08	(-0.24, 0.07)	0.30	0.38	0.05	(-0.07, 0.18)	0.41	0.72
Caudal Volume	0.01	(-0.14, 0.17)	0.86	0.86	0.05	(-0.09, 0.18)	0.48	0.72
Orbitofrontal Cortex								
Medial Volume	-0.10	(-0.25, 0.05)	0.20	0.38	0.06	(-0.07, 0.19)	0.37	0.72
Lateral Volume	-0.13	(-0.29, 0.02)	0.09	0.27	-0.01	(-0.13, 0.12)	0.91	0.91

a. Exposure probability models were fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.

Appendix Table C.5: Marginal models of associations between childhood physical attack, threatened violence exposure, and standardized corticolimbic brain volumes in preadolescence using standardization via the parametric g-formula. n = 2,905. (Marginal Model 2)

	Physical Attack				Threatened Violence			
	Β	95% CI	*p**	*q*	Β	95% CI	*p**	*q*
Amygdala Volume	-0.10	(-0.22, 0.02)	0.10	0.30	0.03	(-0.07, 0.12)	0.59	0.64
Hippocampus Volume	-0.03	(-0.15, 0.09)	0.66	0.73	0.06	(-0.03, 0.15)	0.18	0.54
Anterior Cingulate Cortex								
Rostral Volume	-0.07	(-0.21, 0.06)	0.30	0.45	0.05	(-0.07, 0.16)	0.43	0.64
Caudal Volume	0.03	(-0.11, 0.17)	0.73	0.73	0.05	(-0.07, 0.18)	0.41	0.64
Orbitofrontal Cortex								
Medial Volume	-0.09	(-0.22, 0.03)	0.15	0.30	0.10	(-0.01, 0.22)	0.09	0.54
Lateral Volume	-0.13	(-0.26, 0.00)	0.05	0.30	0.03	(-0.08, 0.14)	0.64	0.64

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.
** P-values calculated after estimation based on bootstrap confidence intervals.*

Appendix Table C.6: Associations between childhood physical attack versus threatened violence exposure and standardized corticolimbic brain volumes in preadolescence in models including only participants exposed to either physical attack or threatened violence, but not to both of them. n = 405. (Direct Comparison Model)

	Physical Attack				Threatened Violence			
Global Measures	Β	95% CI	*p*	*q*	Β	95% CI	*p*	*q*
Amygdala Volume	-0.17	(-0.33, -0.01)	0.04	0.12	0.17	(0.01, 0.33)	0.04	0.12
Hippocampus Volume	-0.12	(-0.28, 0.05)	0.16	0.24	0.12	(-0.05, 0.28)	0.16	0.24
Anterior Cingulate Cortex								
Rostral Volume	-0.11	(-0.31, 0.09)	0.29	0.35	0.11	(-0.09, 0.31)	0.29	0.35
Caudal Volume	-0.02	(-0.23, 0.20)	0.89	0.89	0.02	(-0.20, 0.23)	0.89	0.89
Orbitofrontal Cortex								
Medial Volume	-0.21	(-0.41, -0.01)	0.04	0.12	0.21	(0.01, 0.41)	0.04	0.12
Lateral Volume	-0.14	(-0.34, 0.05)	0.14	0.24	0.14	(-0.05, 0.34)	0.14	0.24

a. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking. Subcortical volume models were additionally adjusted for ICV.
b. q-values were calculated given 3 global measures of brain volume within each exposure via the Simes / Benjamini-Hochberg FDR adjustment method.
c. Because all participants in this subsample experienced one or the other type of violence, effect estimates mirror each other.

Appendix Table C.7. Associations between childhood physical attack exposure, threatened violence exposure, and amygdala volume with and without adjusting for ICV, primary and sensitivity models.

	Physical Attack			Threatened Violence		
	β	95% CI	*p*	β	95% CI	*p*
Primary Model						
ICV unadjusted	-0.17	(-0.30, -0.04)	0.01	0.04	(-0.07, 0.14)	0.49
ICV adjusted	-0.10	(-0.21, 0.01)	0.08	0.03	(-0.06, 0.12)	0.56
Sensitivity Model 1						
ICV unadjusted	-0.19	(-0.36, -0.02)	0.03	0.05	(-0.08, 0.18)	0.47
ICV adjusted	-0.10	(-0.24, 0.05)	0.19	0.01	(-0.10, 0.13)	0.81
Sensitivity Model 2						
ICV unadjusted	-0.18	(-0.31, -0.05)	0.01	0.06	(-0.05, 0.17)	0.27
ICV adjusted	-0.11	(-0.22, 0.00)	0.06	0.04	(-0.05, 0.13)	0.38
Sensitivity Model 3						
ICV unadjusted	-0.18	(-0.33, -0.02)	0.03	0.06	(-0.05, 0.18)	0.29
ICV adjusted	-0.13	(-0.26, 0.01)	0.06	0.03	(-0.07, 0.13)	0.53
Marginal Model 1						
ICV unadjusted	-0.22	(-0.37, -0.07)	0.01	0.00	(-0.12, 0.13)	0.95
ICV adjusted	-0.13	(-0.25, 0.00)	0.05	0.02	(-0.08, 0.11)	0.76
Marginal Model 2						
ICV unadjusted	-0.17	(-0.31, -0.03)	0.02	0.04	(-0.08, 0.15)	0.53
ICV adjusted	-0.10	(-0.22, 0.02)	0.10	0.03	(-0.07, 0.12)	0.59
Direct Comparison Model						
ICV unadjusted	-0.25	(-0.44, -0.06)	< 0.01	0.25	(0.06, 0.44)	<0.01
ICV adjusted	-0.17	(-0.33, -0.01)	0.04	0.17	(0.01, 0.33)	0.04

All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking.

Appendix Table C.8. Associations between childhood physical attack exposure, threatened violence exposure, and hippocampus volume with and without adjusting for ICV, primary and sensitivity models.

	Physical Attack			Threatened Violence		
	Β	95% CI	*p*	Β	95% CI	*p*
Primary Model						
ICV unadjusted	-0.10	(-0.23, 0.04)	0.15	0.07	(-0.03, 0.18)	0.18
ICV adjusted	-0.03	(-0.14, 0.09)	0.64	0.06	(-0.03, 0.15)	0.18
Sensitivity Model 1						
ICV unadjusted	-0.15	(-0.32, 0.03)	0.10	0.08	(-0.04, 0.21)	0.18
ICV adjusted	-0.05	(-0.19, 0.10)	0.54	0.05	(-0.06, 0.15)	0.39
Sensitivity Model 2						
ICV unadjusted	-0.12	(-0.25, 0.02)	0.09	0.09	(-0.02, 0.20)	0.11
ICV adjusted	-0.04	(-0.16, 0.07)	0.48	0.07	(-0.02, 0.16)	0.15
Sensitivity Model 3						
ICV unadjusted	-0.08	(-0.24, 0.09)	0.36	0.11	(-0.01, 0.23)	0.06
ICV adjusted	-0.02	(-0.16, 0.11)	0.74	0.08	(-0.02, 0.18)	0.12
Marginal Model 1						
ICV unadjusted	-0.16	(-0.30, -0.01)	0.04	0.05	(-0.07, 0.17)	0.39
ICV adjusted	-0.06	(-0.19, 0.06)	0.32	0.06	(-0.03, 0.16)	0.17
Marginal Model 2						
ICV unadjusted	-0.10	(-0.23, 0.03)	0.14	0.07	(-0.03, 0.18)	0.18
ICV adjusted	-0.03	(-0.15, 0.09)	0.66	0.06	(-0.03, 0.15)	0.18
Direct Comparison Model						
ICV unadjusted	-0.19	(-0.38, -0.01)	0.04	0.19	(0.01, 0.38)	0.04
ICV adjusted	-0.12	(-0.28, 0.05)	0.16	0.12	(-0.05, 0.28)	0.16

All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking.

Section D. Corporal Punishment Model Results

Appendix Table D.1: Associations between corporal punishment score and standardized brain volumes in preadolescence. n = 2,905

Global Measures	Corporal Punishment			
	ß	95% CI	*p*	*q*
Cortical Gray Matter	-0.07	(-0.11, -0.04)	< 0.01	< 0.01
White Matter	-0.05	(-0.09, -0.01)	< 0.01	< 0.01
Subcortical Gray Matter				
ICV unadjusted	-0.04	(-0.07, 0.00)	0.06	-
ICV adjusted	0.02	(-0.01, 0.04)	0.22	0.22
Corticolimbic ROIs	Corporal Punishment			
	ß	95% CI	*p*	*q*
Amygdala Volume				
ICV unadjusted	-0.05	(-0.09, -0.00)	0.03	-
ICV adjusted	0.00	(-0.04, 0.03)	0.96	0.96
Hippocampus Volume				
ICV unadjusted	-0.04	(-0.08, 0.01)	0.09	-
ICV adjusted	0.01	(-0.03, 0.05)	0.57	0.68
Anterior Cingulate Cortex				
Rostral Volume	-0.08	(-0.13, -0.04)	< 0.01	< 0.01
Caudal Volume	-0.09	(-0.14, -0.05)	< 0.01	< 0.01
Orbitofrontal Cortex				
Medial Volume	-0.06	(-0.10, -0.02)	< 0.01	< 0.01
Lateral Volume	-0.08	(-0.12, -0.04)	< 0.01	< 0.01

a. Corporal punishment score is a continuous sum of responses to 2 items assessing frequency of spanking and slapping, range 0 - 8.

b. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking.

c. q-values were calculated via the Simes / Benjamini-Hochberg FDR adjustment method assuming 3 and 6 global and corticolimbic measures, respectively, of brain volume within each exposure. ICV-unadjusted results were not included in FDR adjustment because they are provided for context only.

Appendix Table D.2: Associations between corporal punishment score, physical attack, and standardized brain volumes in preadolescence in models including both exposure variables simultaneously. n = 2,905.

Global Measures	Corporal Punishment				Physical Attack			
	β	95% CI	*p*	*q*	β	95% CI	*p*	*q*
Cortical Gray Matter	-0.08	(-0.11, -0.04)	< 0.01	< 0.01	-0.15	(-0.27, -0.03)	0.02	0.03
White Matter	-0.05	(-0.09, -0.01)	< 0.01	< 0.01	-0.16	(-0.29, -0.04)	< 0.01	< 0.01
Subcortical Gray Matter								
ICV unadjusted	-0.04	(-0.07, -0.00)	0.05	-	-0.15	(-0.28, -0.03)	0.02	-
ICV adjusted	0.02	(-0.01, 0.04)	0.23	0.23	-0.05	(-0.14, 0.03)	0.24	0.24

Corticolimbic ROIs	Corporal Punishment				Physical Attack			
	β	95% CI	*p*	*q*	β	95% CI	*p*	*q*
Amygdala Volume								
ICV unadjusted	-0.05	(-0.09, -0.01)	0.02	-	-0.26	(-0.42, -0.09)	< 0.01	-
ICV adjusted	-0.00	(-0.04, 0.03)	0.89	0.89	-0.20	(-0.34, -0.05)	< 0.01	< 0.01
Hippocampus Volume								
ICV unadjusted	-0.04	(-0.08, 0.01)	0.09	-	-0.10	(-0.26, 0.07)	0.26	-
ICV adjusted	0.01	(-0.03, 0.05)	0.58	0.70	-0.03	(-0.18, 0.11)	0.66	0.79
Anterior Cingulate Cortex								
Rostral Volume	-0.09	(-0.13, -0.04)	< 0.01	< 0.01	-0.10	(-0.27, 0.08)	0.27	0.41
Caudal Volume	-0.09	(-0.14, -0.05)	< 0.01	< 0.01	0.02	(-0.16, 0.20)	0.81	0.81
Orbitofrontal Cortex								
Medial Volume	-0.06	(-0.10, -0.02)	< 0.01	< 0.01	-0.12	(-0.29, 0.04)	0.15	0.32
Lateral Volume	-0.08	(-0.12, -0.04)	< 0.01	< 0.01	-0.12	(-0.29, 0.05)	0.16	0.32

a. Corporal punishment score is a continuous sum of responses to 2 items assessing frequency of spanking and slapping, range 0 - 8.

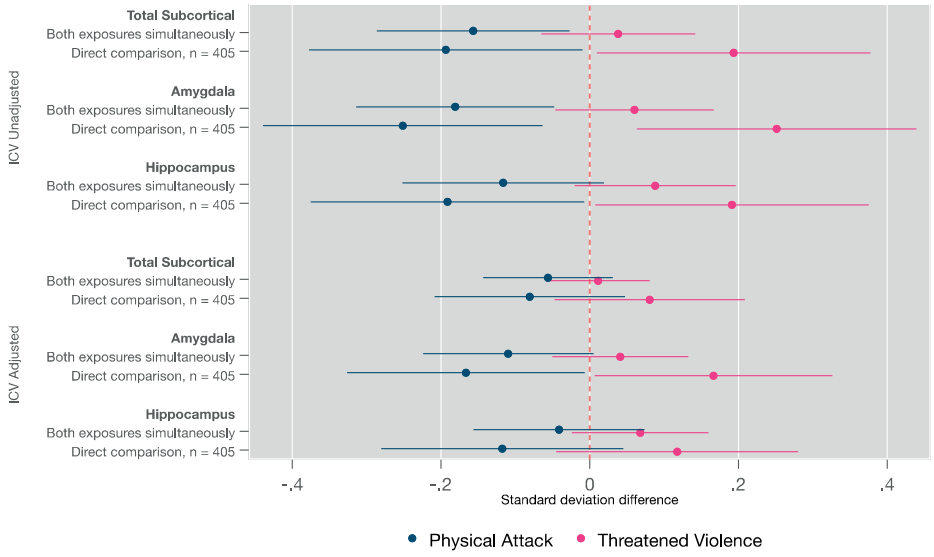
b. All models are fully adjusted and include covariates for child age at MRI scan, sex, and ethnicity; household income at birth; highest parental education level achieved; maternal and paternal history of psychosis; maternal and paternal psychopathology symptoms; maternal age at the child's birth; and child in utero exposure to smoking.

c. q-values were calculated via the Simes / Benjamini-Hochberg FDR adjustment method assuming 3 and 6 global and corticolimbic measures, respectively, of brain volume within each exposure. ICV-unadjusted results were not included in FDR adjustment because they are provided for context only.

Section E. Selected Sensitivity Model Results for Subcortical Brain Volumes

Figure E.1

Subcortical Volumes: Comparison of Selected Effect Estimates



Appendix Figure E.1. Associations between physical attack (navy), threatened violence (pink), and standardized subcortical brain volumes in selected sensitivity models.

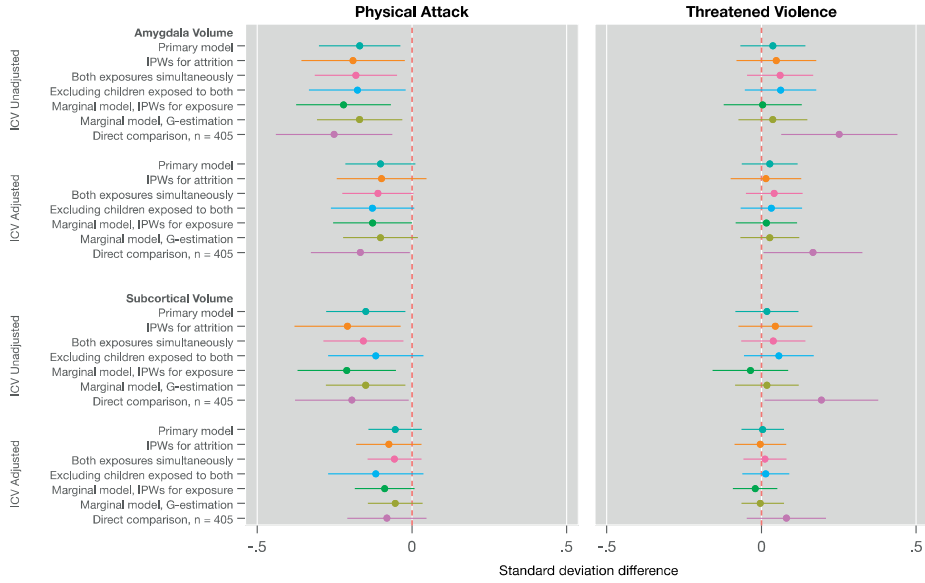
Models with "both exposures simultaneously" include covariates for both actual and mere threatened violence exposure simultaneously (Sensitivity Model 2). n = 2,905.

Direct Comparison models use a subsample of participants exposed to either physical attack or threatened violence, but not to both of them. n = 405. Because all participants in this subsample experienced one or the other type of violence, effect estimates mirror each other.

Estimates are from fully adjusted models accounting for child scan age, sex, ethnicity, household income at birth, highest parental education level achieved, maternal and paternal history of psychosis, maternal and paternal psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.

Figure E.2

Subcortical Brain Volumes: Primary and Sensitivity Model Estimates, Unadjusted and Adjusted for ICV



Appendix Figure E.2. Associations between physical attack, threatened violence, and selected standardized subcortical volumes using multiple modeling strategies. All models use sample size n = 2,905 unless otherwise stated.

Primary models are OLS-estimated linear regression models in the full analytic sample. n = 2,905.

Models using "IPWs for attrition" use inverse probability of attrition weights to account for selection bias (Sensitivity Model 1).

Models with "both exposures simultaneously" include covariates for both actual and mere threatened violence exposure simultaneously (Sensitivity Model 2).

Models "excluding children exposed to both" exclude participants exposed to both actual and mere threatened violence (Sensitivity Model 3). n = 2,570 for physical attack; n = 2,703 for threatened violence.

Marginal models using "IPWs for exposure" are fit using inverse probability of exposure weights.

Marginal models using G-Estimation are fit using standardization via the parametric G-formula.

Direct Comparison models use a subsample of participants exposed to either physical attack or threatened violence, but not to both of them. n = 405.

Estimates are from fully adjusted models accounting for child scan age, sex, ethnicity, household income at birth, highest parental education level achieved, maternal and paternal history of psychosis, maternal and paternal psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.

Appendix References

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6

Poverty from fetal life onward and child brain morphology: differential association by minority status

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ABSTRACT

Poverty is a risk factor for impaired child development, an association possibly mediated by brain morphology. Previous studies lacked prospective poverty assessments during pregnancy and did not stratify by majority/minority status. We investigated the association of household poverty from fetal life forward with brain morphological differences at age 10 years, in 2166 mother-child dyads. Children ever exposed to poverty had smaller amygdala volumes, especially if exposed in pregnancy. Importantly, the associations differed by majority/minority status. Of the children from non-European minority descent, those exposed to poverty had smaller amygdala volumes than non-exposed minority controls, suggesting a role of the stress response system. In contrast, children from Dutch majority group ever-exposed to poverty had smaller global brain volumes than majority controls, reflecting broad developmental disadvantages. The smaller total brain volume mediated the association between poverty and poorer school performance. Our findings suggest different mechanisms and vulnerabilities across majority and minority groups.

INTRODUCTION

Poverty is a well-known determinant of numerous dimensions of child development (Duncan & Brooks-Gunn, 2000). In addition to poor physical development, impaired cognitive functions and socioemotional development consistently occur more often in children exposed to poverty (Bradley & Corwyn, 2002). Child brain development has been examined as a neurobiological factor possibly mediating these associations (Hair et al., 2015; Whittle et al., 2017). Poverty is related to brain developmental disadvantages due to deprivation of cognitive stimulation, inadequate nutrition, exposure to environmental toxins and psychological stress (Hackman et al., 2010), which perpetuate structural inequalities in society (Marmot et al., 2008). Most studies reported positive associations between income and total gray and white matter volumes (Hair et al., 2015; Hanson et al., 2013; Luby et al., 2013), indicating that poverty and structural deprivation have a global impact on brain development, possibly as part of stunted growth. Other research on child exposure to low income (Hair et al., 2015; Luby et al., 2013; Raffington et al., 2019; Whittle et al., 2017) focused on regions of interest, in particular the hippocampus and amygdala. These studies are conducted against the background that these subcortical structures, which are rich in cortisol receptors, are more sensitive to stress (Tottenham & Sheridan, 2009). Studies examining poverty and the hippocampal and amygdala volumes yielded mixed findings, with some reporting smaller volumes of the hippocampus (Hair et al., 2015; Luby et al., 2013; Raffington et al., 2019) and amygdala (Luby et al., 2013; Noble et al., 2012; Whittle et al., 2017) and others no association with the hippocampus (Whittle et al., 2017) and amygdala (Hair et al., 2015; Noble et al., 2015). These inconsistent findings might be due to small sample sizes (Betancourt et al., 2016; Hair et al., 2015; Hanson et al., 2013; Luby et al., 2013; Noble et al., 2012; Raffington et al., 2019; Whittle et al., 2017). In addition, only few studies were conducted outside of the US (Jednoróg et al., 2012; Raffington et al., 2019; Whittle et al., 2017). The US and Western European countries are different in terms of welfare policy (Caminada & Martin, 2011), the level of inequality (Alvaredo et al., 2018) and poverty rate (OECD, 2020); hence the impact of poverty may differ and studies in non-US countries are important to explore generalizability of results.

A few studies examined whether brain morphology mediated the association between income and cognitive functions (Hair et al., 2015; Noble et al., 2015). In a large cross-sectional study of 389 participants aged 4 to 22 years, those from low-income household scored lower on IQ tests than those from high- or middle-income households, and approximately 20% of this association could be explained by smaller volumes of the frontal and temporal lobes (Hair et al., 2015). Similarly, in individuals aged between 3 and 20 years, whole-brain surface area partially accounted for the association between household income and executive functions (Noble et al., 2015). These studies were

cross-sectional and the statistical mediation models can thus not be interpreted well. Prospective studies are needed to evaluate whether important functional consequences of low household family income, such as less optimal offspring cognitive function, are explained by differences in brain morphology.

Brain development starts rapidly prenatally, and although it continues beyond adolescence, the volumes of many structures already approach their maximum volume 2 years after birth (Lenroot & Giedd, 2006). The different developmental trajectories of each region (Belsky & de Haan, 2011; Lenroot & Giedd, 2006) could underlie a differential impact of prenatal and postnatal poverty. Also, critical brain developmental processes, such as the neuronal migration and gyrification, occur primarily during the prenatal period (White, 2019). Thus, exposure to adverse conditions in fetal life, such as famine, could have long-term implications (White, 2019). Children institutionalized from birth showed smaller hippocampal volumes, which was followed by catch-up only among those placed in higher quality care before 18 months old (Fox et al., 2010; Tottenham & Sheridan, 2009). These reports support a critical period of brain development from fetal period to infancy. However, little is known about the role of timing in the association between poverty and brain morphology since most studies in childhood or adolescence were cross-sectional.

Importantly, minority status and poverty co-occur in many societies (Cheng & Goodman, 2015). Minority populations often experience institutional and cultural discrimination (e.g. residential segregation and negative stereotypes), which can lead to differences in socioeconomic status (Williams & Mohammed, 2013; Williams et al., 2010). Some scholars argue that racial disparities in health largely reflect differences in socioeconomic status between majority and minority populations, yet racial health disparities often remain after taking socioeconomic status into account (Williams et al., 2010). Others argue that minority status and poverty interact in the relation with poor health outcomes (Bauer, 2014). In migrants, poverty status may be tied to inequity and discrimination, and the resulting stress that can impact child development may be greater than in majority groups (Myers, 2009). A previous study from our current cohort showed associations between exposure to prenatal stress and offspring IQ only in ethnic minorities (Cortes Hidalgo et al., 2020). Therefore, examining whether there are differences in the association between poverty and brain morphology by majority and minority status is critical but, to the best of our knowledge, has not been done.

In the current study, we investigated the association between exposure to poverty, defined as living in a family with household income below the national low-income threshold, and child brain morphology. In line with previous findings of an association between poverty and global brain metrics (Betancourt et al., 2016; Hair et al., 2015; Hanson et al., 2013; Luby et al., 2013; Noble et al., 2012), we hypothesized that poverty would be associated with smaller total brain, cortical gray matter, and cerebral white

matter volumes. Next, we examined the association between exposure to poverty and child brain morphology by timing of poverty exposure. The timing of exposure was categorized into prenatal period and early childhood (postnatal period) within critical period (i.e. first 5 years of life). We hypothesized that prenatal exposure to poverty is more strongly associated with differences in brain morphology than postnatal exposure. Also, we hypothesized that poverty may be differentially associated with these structural brain differences in majority and minority groups. Further, we examined whether the association of exposure to poverty with brain morphology explained some differences in later cognitive functions as captured by school performance.

RESULTS

Data from the Generation R Study, a prospective population-based birth cohort in Rotterdam, the Netherlands, was analyzed (Jaddoe et al., 2012). In total, 5311 pregnant women provided data on standardized household income in pregnancy. After excluding those without data on poverty status and brain magnetic resonance imaging (MRI), and keeping one of two siblings, a total of 2166 children were left for the analytical sample (Figure 1). Children of low socioeconomic status and minority status tended to be lost to follow-up (Supplementary Table 1), but characteristics were not critically different between those excluded and included. The correlations among variables of interests in the current study are shown in Figure 2.

Poverty was defined by the household standardized income, calculated using family size and household income, under the national low-income threshold of the Netherlands (e.g. (*Armoedebericht*)). Of all children, 20.4% (n = 442) were in poverty in one or more assessment periods (Table 1): 5.1% were poor in pregnancy only, 5.4% in childhood only (when children were 3 and 5 years old) and 9.9% in both periods. Minority was defined according to maternal national origin following definitions used by Statistics Netherlands (*Statistical Yearbook of the Netherlands 2004*, 2004). The Netherlands do not use a race categorizations but parental national origin to denote recent immigration. We collapsed these to "Dutch", "Non-Dutch Western", and "Non-Western"; the latter included Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, other African, middle and other south American and most Asian origins. Only 115 of 1250 (9.2%) children from Dutch majority group, but 297 of 530 (56.0%) children from non-Western minority group have ever experienced poverty. The group of children that experienced poverty only in childhood included 58 children of Dutch majority status (50.0%) and 52 children of non-Western minority status (44.8%). The sample characteristics by majority and minority statuses are available in Supplementary Table 2.

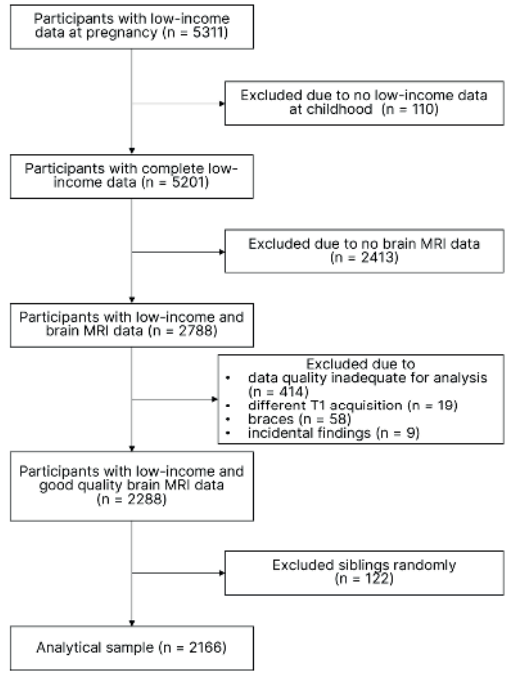

Figure 1. Sampling flow chart

During pregnancy, experience of discrimination related to ethnicity was measured among minority population. Minority mothers who were exposed to poverty reported more discrimination (mean = 3.99, SD = 3.6, assessed before the birth of the child) than those had not exposed to poverty (mean = 2.55, SD = 3.1) (B = 0.76, 95%CI = 0.11; 1.42; adjusted for maternal IQ, maternal educational attainment, and maternal and paternal psychiatric symptoms), demonstrating a link between poverty and the experience of ethnic discrimination among minority populations.

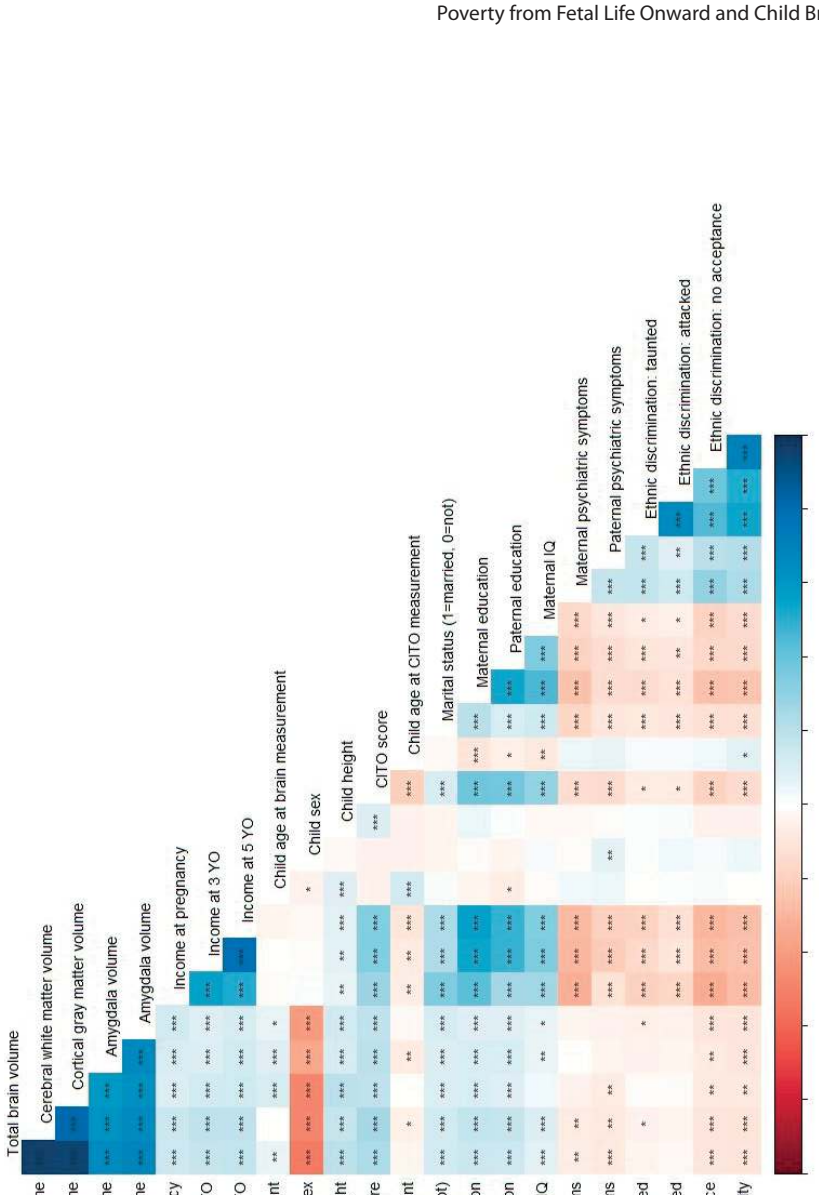

Figure 2. Correlation matrix of child brain morphology, household income, and child and familial demographic characteristics
The color grading gives the correlation strengths. Complete cases were analyzed.
* indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$.

Table 1. Sample characteristics (N = 2166)

Characteristics	Never poverty N = 1724 (79.6%)		Ever poverty N = 442		Timing of poverty exposure					
					Poverty in pregnancy only N = 111 (5.1%)		Poverty in childhood only N = 116 (5.4%)		Chronic poverty N = 215 (9.9%)	
Child sex										
Male, N, %	843	48.9	215	48.6	51	45.9	57	49.1	107	49.8
Female, N, %	881	51.1	227	51.4	60	54.1	59	50.9	108	50.2
Child age at MRI measurement (years), mean, SD	10.1	0.6	10.2	0.6	10.2	0.6	10.2	0.6	10.1	0.5
Child school performance (CITO score), mean, SD	539.9	7.7	534.0	9.2	536.2	9.0	534.3	8.9	532.7	9.3
Maternal ethnicity										
Dutch, N, %	1250	72.5	115	26.0	22	19.8	58	50.0	35	16.3
Non-Dutch Western, N, %	241	14.0	30	6.8	12	10.8	6	5.2	12	5.6
Non-Western, N, %	233	13.5	297	67.2	77	69.4	52	44.8	168	78.1
Maternal education at pregnancy [a]										
High, N, %	681	39.5	17	3.9	7	6.5	6	5.2	4	1.9
Mid-high, N, %	484	28.6	68	15.3	19	17.4	28	23.6	21	9.8
Mid-low, N, %	437	25.3	169	38.2	44	39.4	54	46.9	71	32.8
Low, N, %	112	6.5	188	42.6	41	36.6	28	24.3	119	55.6
Maternal IQ [b]**, mean, SD**	102.0	12.5	90.1	15.0	95.1	13.1	93.8	14.7	85.6	14.7
Parental psychiatric symptoms at pregnancy										
Mother [c], median, interquartile range	0.12	0.2	0.27	0.5	0.23	0.4	0.19	0.3	0.40	0.6
Father [d], median, interquartile range	0.06	0.1	0.12	0.2	0.08	0.2	0.06	0.2	0.18	0.3

The data was combined across imputed datasets.

Non-Dutch Western includes Indonesian, American, Asian, European, Oceanian. Non-Western includes Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, African, American non-Western, Asian non-Western.

Ever poverty is a total of “poverty in pregnancy only”, “poverty in childhood only” and “chronic poverty “.

a: missing data N = 54 (2.5%)

b: missing data N = 138 (6.4%)

c: missing data N = 233 (10.8%)

d: missing data N = 583 (26.9%)

Poverty and child brain morphology by timing of poverty exposure

Child brain morphological data were collected when children were approximately at the age of 10.1 (SD: 0.6). The association between poverty experience and brain morphology was examined, adjusting for child age and sex, minority or majority status, maternal IQ, maternal educational attainment, and maternal and paternal psychiatric symptoms. We observed no association between exposure to poverty at any assessment timing and the global child brain morphology measures in the total sample (e.g. total brain volume: B = -0.10, 95%CI = -0.21; 0.01) (Table 2). Likewise, poverty in pregnancy only, poverty in childhood only and chronic poverty statuses were not associated with global child brain morphology. However, the exposure to poverty was associated with child subcortical brain morphology (Table 3). Children ever exposed to poverty had smaller amygdala volumes (B = -0.11, 95%CI = -0.21; -0.002). In particular, children experiencing poverty in pregnancy (which included the chronically exposed group) had smaller amygdala volumes (in pregnancy only: B = -0.18, 95%CI = -0.34; -0.02, chronically: B = -0.17, 95%CI = -0.31; -0.03). We combined these two groups experiencing poverty in pregnancy (any pregnancy exposure: B = -0.17, 95%CI = -0.29; -0.06). The lack of overlap in 84%CIs of the associations between any poverty exposure and amygdala volumes (poverty in pregnancy: -0.26; -0.09, poverty in childhood only: -0.08; 0.13) provides statistical evidence for a differential association by timing of exposure (Julious, 2004).

As a sensitivity analysis, child height was added to the model to examine possible stunting as an indicator of general physical development. Child height was measured approximately 1-2 months prior to brain measurement. Further adjustment for age-standardized child height did not meaningfully change results. Also, sex interaction with exposure to poverty was examined to assess the robustness of the findings for both girls and boys. We found no interaction effect by child sex (Supplementary Table 3).

Table 2. The association of poverty with global brain morphology (N = 2166)

		Total brain volume			Cortical gray matter volume			Cerebral white matter volume		
	N	B	95%CI	P-value	B	95%CI	P-value	B	95%CI	P-value
Model 1										
Never-low-income	1724	0	Ref.		0	Ref.		0	Ref.	
Ever-low-income	442	-0.16	-0.26; -0.06	<0.01	-0.18	-0.28; -0.08	<0.01	-0.13	-0.23; -0.03	0.02
Low-income-in-pregnancy-only	111	-0.08	-0.25; 0.09	0.34	-0.13	-0.30; 0.04	0.14	-0.03	-0.21; 0.14	0.72
Low-income-in-childhood-only	116	-0.20	-0.36; -0.04	0.02	-0.18	-0.34; -0.02	0.03	-0.19	-0.35; -0.02	0.02
Chronic-low-income	215	-0.19	-0.32; -0.05	0.01	-0.20	-0.34; -0.07	0.01	-0.14	-0.27; -0.01	0.04
Model 2										
Never-low-income		0	Ref.		0	Ref.		0	Ref.	
Ever-low-income		-0.10	-0.21; 0.01	0.06	-0.11	-0.22; 0.004	0.06	-0.09	-0.20; 0.02	0.10
Low-income-in-pregnancy-only		-0.03	-0.20; 0.14	0.76	-0.07	-0.24; 0.11	0.46	0.004	-0.17; 0.18	0.97
Low-income-in-childhood-only		-0.14	-0.30; 0.02	0.09	-0.12	-0.28; 0.05	0.16	-0.16	-0.32; 0.01	0.07
Chronic-low-income		-0.12	-0.26; 0.03	0.11	-0.12	-0.27; 0.02	0.10	-0.10	-0.25; 0.05	0.18
Model 3										
Never-low-income		0	Ref.		0	Ref.		0	Ref.	
Ever-low-income		-0.10	-0.21; 0.01	0.08	-0.11	-0.22; 0.01	0.06	-0.09	-0.20; 0.03	0.14
Low-income-in-pregnancy-only		-0.03	-0.20; 0.15	0.76	-0.07	-0.25; 0.11	0.43	0.01	-0.17; 0.18	0.96
Low-income-in-childhood-only		-0.14	-0.31; 0.02	0.08	-0.12	-0.28; 0.05	0.16	-0.16	-0.33; 0.01	0.06
Chronic-low-income		-0.11	-0.26; 0.05	0.17	-0.12	-0.27; 0.03	0.12	-0.08	-0.24; 0.07	0.30

Model 1 adjusted for child age at brain measurement, child sex and maternal ethnicity.
Model 2: model 1 + maternal IQ and maternal educational attainment at pregnancy.
Model 3: model 2 + maternal and paternal psychiatry symptoms at pregnancy.
All brain measures of outcome are standardized.
Ever poverty is a total of “poverty in pregnancy only”, “poverty in childhood only” and “chronic poverty “.

Table 3. The association of poverty with subcortical regional brain morphology (N = 2166)

		Mean hippocampus volume			Mean amygdala volume		
	N	B	95%CI	P-value	B	95%CI	P-value
Model 1							
Never-low-income	1724	0	Ref.		0	Ref.	
Ever-low-income	442	-0.03	-0.12; 0.07	0.61	-0.07	-0.16; 0.03	0.17
Low-income-in-pregnancy-only	111	-0.10	-0.26; 0.06	0.22	-0.14	-0.30; 0.02	0.09
Low-income-in-childhood-only	116	-0.06	-0.21; 0.09	0.43	0.05	-0.10; 0.20	0.48
Chronic-low-income	215	0.04	-0.09; 0.17	0.52	-0.11	-0.23; 0.02	0.09
Model 2							
Never-low-income		0	Ref.		0	Ref.	
Ever-low-income		-0.03	-0.13; 0.07	0.59	-0.10	-0.20; 0.001	0.05
Low-income-in-pregnancy-only		-0.10	-0.26; 0.06	0.22	-0.17	-0.33; -0.01	0.04
Low-income-in-childhood-only		-0.06	-0.21; 0.09	0.44	0.03	-0.13; 0.18	0.74
Chronic-low-income		0.05	-0.09; 0.18	0.50	-0.16	-0.29; -0.02	0.02
Model 3							
Never-low-income		0	Ref.		0	Ref.	
Ever-low-income		-0.05	-0.15; 0.05	0.35	-0.11	-0.21; -0.002	0.05
Low-income-in-pregnancy-only		-0.12	-0.28; 0.04	0.15	-0.18	-0.34; -0.02	0.03
Low-income-in-childhood-only		-0.06	-0.22; 0.09	0.41	0.02	-0.13; 0.18	0.75
Chronic-low-income		0.01	-0.13; 0.15	0.87	-0.17	-0.31; -0.03	0.02

Model 1 adjusted for child age at brain measurement, child sex, maternal ethnicity, and total intracranial volume.
Model 2: model 1 + maternal IQ and maternal educational attainment at pregnancy.
Model 3: model 2 + maternal and paternal psychiatry symptoms at pregnancy.
All brain measures of outcome are standardized.
Ever poverty is a total of “poverty in pregnancy only”, “poverty in childhood only” and “chronic poverty “.

Poverty and child brain morphology by majority and minority status

Next, we stratified the association by majority and minority statuses (Table 4). The interaction effect by majority and minority statuses was significant for the total brain and cerebral white matter volumes (p for interaction = 0.05 and 0.04, respectively) (Supplementary Table 4). In children of Dutch majority group, poverty exposure was associated with smaller total brain (B = -0.21, 95%CI = -0.38; -0.04), cortical gray matter (B = -0.18, 95%CI = -0.36; -0.01) and cerebral white matter volumes (B = -0.22, 95%CI = -0.40; -0.05). These associations were most obvious if exposure occurred in childhood. In contrast, among minority children, exposure to poverty at any assessment time was not associated with global brain volumes, e.g. total brain volume (B = -0.02, 95%CI = -0.20; 0.15). However, having ever been exposed to poverty was associated with smaller amygdala volumes (B = -0.15, 95%CI = -0.31; 0.01), especially if the exposure was in pregnancy (B = -0.21, 95%CI = -0.37; -0.04). This association of pregnancy exposure to poverty and less amygdala volume was also observed in the majority children exposed to poverty only in pregnancy, but did not reach significance (any exposure in pregnancy: B = -0.18, 95%CI = -0.40; 0.04). However, few majority group children were exposed to poverty in pregnancy (22 in pregnancy only and 35 both in pregnancy and childhood, the respective numbers in minority children were 77 and 168; although more than twice as many children of Dutch majority group have participated). No association with hippocampal volume was found in either group. The brain morphologies that differed by poverty status are shown in Figure 3. This illustrates that the volume smaller in minority children exposed to poverty (i.e. amygdala volume; shown in red) is relatively small compared to the total brain volume associated with poverty exposure in majority children (shown in blue).

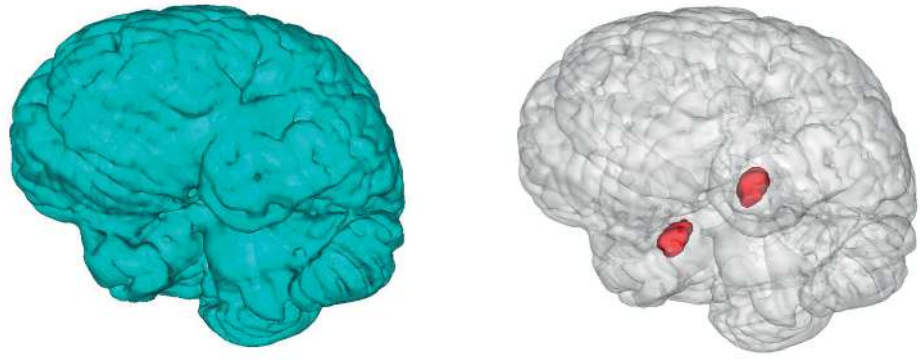

Figure 3. T1-weighted MRI scan showing the total brain (in blue) and amygdala (in red)

Table 4. The association between timing of poverty exposure and brain morphology among children of Dutch majority and non-Western minority ethnic groups

		Total brain volume			Cortical gray matter volume			Cerebral white matter volume			Mean hippocampus volume			Mean amygdala volume		
	N	B	95%CI	P-value	B	95%CI	P-value	B	95%CI	P-value	B	95%CI	P-value	B	95%CI	P-value
Dutch (N = 1365)																
Never-low-income	1250	0	Ref.		0	Ref.		0	Ref.		0	Ref.		0	Ref.	
Ever-low-income	115	-0.21	-0.38; -0.04	0.01	-0.18	-0.36; -0.01	0.04	-0.22	-0.40; -0.05	0.01	0.03	-0.13; 0.19	0.68	-0.07	-0.23; 0.09	0.40
In pregnancy only	22	-0.12	-0.48; 0.24	0.52	-0.17	-0.54; 0.20	0.36	-0.07	-0.43; 0.30	0.73	-0.07	-0.41; 0.27	0.69	-0.27	-0.60; 0.07	0.12
In childhood only	58	-0.23	-0.45; 0.001	0.05	-0.17	-0.40; 0.07	0.16	-0.25	-0.48; -0.01	0.04	-0.02	-0.23; 0.19	0.86	0.04	-0.17; 0.25	0.72
Chronic	35	-0.25	-0.55; 0.05	0.10	-0.21	-0.52; 0.09	0.17	-0.28	-0.59; 0.02	0.07	0.20	-0.08; 0.48	0.16	-0.12	-0.40; 0.15	0.38
Non-Western (N = 530)																
Never-low-income	233	0	Ref.		0	Ref.		0	Ref.		0	Ref.		0	Ref.	
Ever-low-income	297	-0.02	-0.20; 0.15	0.79	-0.07	-0.24; 0.11	0.46	0.02	-0.17; 0.20	0.87	-0.12	-0.28; 0.03	0.12	-0.15	-0.31; 0.01	0.06
In pregnancy only	77	0.05	-0.18; 0.28	0.68	-0.01	-0.24; 0.22	0.93	0.09	-0.15; 0.33	0.45	-0.16	-0.37; 0.05	0.14	-0.20	-0.41; 0.004	0.05
In childhood only	52	-0.04	-0.30; 0.22	0.77	-0.05	-0.31; 0.21	0.71	-0.05	-0.32; 0.22	0.74	-0.11	-0.35; 0.13	0.38	0.01	-0.23; 0.25	0.93
Chronic	168	-0.07	-0.28; 0.14	0.52	-0.12	-0.33; 0.10	0.28	-0.01	-0.23; 0.22	0.96	-0.11	-0.30; 0.09	0.28	-0.21	-0.40; -0.02	0.03

Model for Dutch adjusted for child age at brain measurement, child sex, maternal education at pregnancy, maternal IQ, maternal and paternal psychiatric symptoms at pregnancy.

Model for non-Western adjusted for covariates in the model for Dutch + detailed maternal ethnicity (Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, African, middle and south American and Asian (except for Indonesian and Japanese)).

Model for hippocampus and amygdala volumes additionally adjusted for total intracranial volume.

All brain measures of outcome are standardized.

Ever poverty is a total of “poverty in pregnancy only”, “poverty in childhood only” and “chronic poverty “.

Poverty, child brain morphology, and school performance

Next, we examined whether the association between exposure to poverty and smaller global brain volumes in majority children underlies cognitive functions. Child cognitive functions were measured via the CITO test (van der Lubbe, 2018), the most common mandatory academic examination conducted in primary school at a mean age of 12 years, which guides the choice for secondary education. In the current sample, CITO score was collected when children were approximately at age 11.9 (SD: 0.4). The test score was standardized, ranging from 500 to 550, with higher scores indicating higher cognitive functions. After we confirmed the association between poverty and cognitive functions (B = -3.05, 95%CI = -4.44; -1.66), and between total brain volume and cognitive functions with multivariate linear regression (B = 1.80, 95%CI = 1.37; 2.23), causal mediation analysis was performed (Tingley et al., 2014). Difference in total brain volume explained the association between exposure to poverty and cognitive functions as the indirect effect accounted for 12% of the total effect (indirect effect: B = -0.36, 95%CI = -0.66; -0.05) (Figure 4). This demonstrates that smaller total brain volumes partially account for the association between living in poor household and less optimal school performance in Dutch majority children.

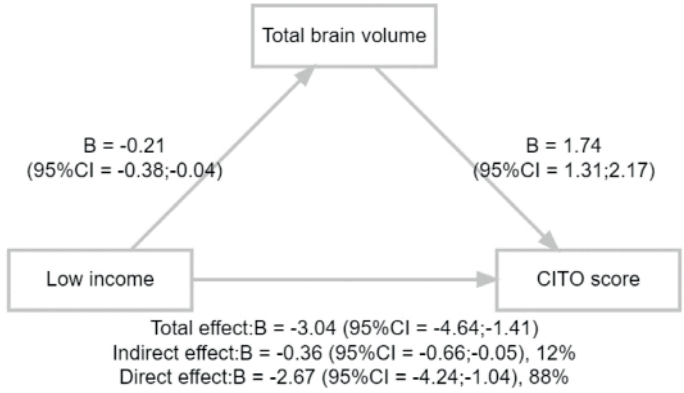

Figure 4. Mediating role of total brain volumes on the association between exposure to poverty and school performance in children from Dutch majority group.
Total sample: n = 1365.
Model adjusted for: poverty → total brain volume: child age at brain measurement, child sex, maternal education at pregnancy, maternal IQ, maternal and paternal psychiatric symptoms at pregnancy; total brain volume → school performance: child age at CITO assessment, child age at brain measurement, child sex, maternal education at pregnancy, maternal IQ, maternal and paternal psychiatric symptoms at pregnancy; poverty → school performance: child age at CITO assessment, child sex, maternal education at pregnancy, maternal IQ, maternal and paternal psychiatric symptoms at pregnancy.

DISCUSSION

We found that exposure to poverty was associated with child brain morphology at age 10 years, and this association differed across majority and minority groups. Overall, children ever exposed to poverty had smaller amygdala volume, but this finding was mainly accounted for by the prenatal exposure of minority children. In these children of non-Western minority group, we also found an association between poverty and ethnic discrimination: mothers who were below the national low-income threshold reported more discrimination during pregnancy. In the Dutch majority children, exposure to poverty was related to smaller total brain, cortical gray matter, and cerebral white matter volumes; associations not found in minority children. Mediation analysis revealed that this association of exposure to poverty on total brain volume in the Dutch underlies some differences in school performance. These findings are an important addition to the literature for several reasons. We prospectively assessed poverty exposure from pregnancy onward and thus prior to brain assessment. This not only enabled us to infer temporal associations more reliably but to study the importance of timing of poverty experience. Further, our study comprised the largest sample outside of the US including participants of multiple national origins, which allowed us to assess differences between majority and minority groups. Importantly, we analyzed the association between poverty exposure and the preadolescent brain morphology also in relation to cognitive functions assessed after the neuroimaging.

Most studies report some association between poverty and brain characteristics, but the evidence for an association with specific regional child brain morphology is mixed; this inconsistency also pertains to the amygdala. The mixed findings may be due to differences in the choice of adjustment strategies, or the age at brain scanning. A study assessing 1099 three-to-twenty-years-old people showed no cross-sectional association between income and volumes of total white matter, hippocampus, and amygdala (Noble et al., 2015). In contrast, a longitudinal study found an association between lower income-to-need ratio and smaller cortical gray and white matter, hippocampus, and amygdala volumes (Luby et al., 2013), similar to our results in the partially-adjusted models. We conducted all brain imaging of participants in a narrow age interval and adjusted for several confounders, providing more reliable estimates. Our results highlight two additional explanations for the seemingly inconsistent findings that will be discussed below. First, we stratified by majority/minority status as in minority groups discrimination and poverty often co-occur. Second, we addressed the timing of exposure, while most childhood studies included a wide age range of poverty experience and did not distinguish between periodic and chronic poverty.

The current study is the first to prospectively examine differential associations of poverty experience with child brain morphology by developmental periods. We showed

that the difference in amygdala volume related to low income was more pronounced if the exposure occurred in pregnancy, a critical brain developmental period (Lenroot & Giedd, 2006). During the prenatal period, the fetal brain undergoes the greatest growth including the neuronal migration and gyrification, and the total number of neurons for the lifetime is created (White, 2019). Previous research have shown some supporting findings: an association between prenatal stress, indexed by intrauterine concentration of cortisol (Buss et al., 2012) or interleukine-6 (Graham et al., 2018), and offspring amygdala volumetric differences; and an association of poverty exposure right after birth with lower total and subcortical gray matter volumes in infancy (Betancourt et al., 2016).

Our study also revealed differences in the association by majority/minority status. Among non-Western minority children, being ever exposed to poverty was associated with smaller amygdala volumes. We speculate that exposure to poverty may be differentially experienced by families of non-Western minority group for several reasons. First, these families may have less material resources and social support. Second, they may have more problems to navigate the social welfare system. Third, we showed that minority mothers of poor households reported more acculturation difficulties and discrimination related to their ethnicity than mothers of non-poor minority households during pregnancy. These experiences likely result in stress, and this stress exposure during pregnancy could specifically affect the development of vulnerable fetal brain regions, like the amygdala. The amygdala has a large number of cortisol receptors (Tottenham & Sheridan, 2009), thus stress induced by poverty status may lead to smaller amygdala volume through prolonged activation and exhaustion (Tottenham & Sheridan, 2009). However, in the absence of a biological stress measure, we cannot demonstrate that the association between poverty in pregnancy and smaller amygdala volume of minority children is explained by stress specific to minorities. A study from the current cohort has found an association between self-reported prenatal maternal stress and offspring IQ only among ethnic minorities (Cortes Hidalgo et al., 2020). Our findings in non-Western children accounted for the association between poverty exposure and smaller amygdala volume in the overall sample. However, the distribution of poverty-exposed children differed between the majority and minority groups: non-Western children were mostly exposed in pregnancy or chronically, whereas few Dutch children were exposed in pregnancy.

Children of Dutch majority with poverty exposure showed smaller total brain, cortical gray matter, and cerebral white matter volumes. This association was not found in children with non-Western minority group, further supporting heterogeneous associations between poverty and brain morphology by majority and minority status. The smaller global brain volumes in children of Dutch majority group exposed to poverty might be indicative of cumulative exposure to neurodevelopmental burden due to socioeconomic disadvantage, poor diet, structural deprivation, and less familial

reserves. However, adjustment for child height, another indicator of global thriving, did not change results and provided no support for a stunting hypothesis, suggesting that the association might be specific to the brain. The lack of association with global brain measures in non-Western minority children may suggest that - although experiencing discrimination - minorities have familial or other resilience factors that reduce its impact on broader neurodevelopment (Sarkisian & Gerstel, 2004; Taylor et al., 2015).

The differences in global brain morphology in majority children mediated the association between poverty and later school performance, such that those exposed to poverty had a lower CITO score (i.e. school performance) that could be accounted for by a smaller total brain volume. This was in concordance with previous findings on the mediating role of volumes of frontal and temporal lobe on the association between poverty and child IQ (Hair et al., 2015); likely, whole-brain surface area partially accounted for the association between household income and executive functions (Noble et al., 2015). Our study adds to this evidence, suggesting that poverty from fetal life to first 5 years of life was associated with later child school performance through a potential impact on brain morphology. This may also shed some light on the intergenerational transmission of poverty via offspring brain development early in life as school performance is related to later socioeconomic success.

Our study had several limitations. First, a substantial number of participants did not undergo the imaging procedure. This decreased the power and introduced a bias, as people from lower socioeconomic backgrounds were more susceptible to loss to follow-up. Second, poverty status might be misclassified since income was self-reported. However, the official poverty prevalence in Rotterdam was similar (*Armoedebericht*). Third, we measured brain morphology at one time point. Considering that brain developmental trajectories show an inverse U-shape (Lenroot & Giedd, 2006), we cannot confirm whether smaller volumes reflect delayed or accelerated development. However, given the age of our sample (9-11 years), most structures will not have started to decrease in volume yet.

In conclusion, our findings support an association between early-life poverty exposure and preadolescent brain morphology. Specifically, we found differential associations across majority and minority groups, suggesting that minority groups may be impacted by poverty-related stress including ethnic discrimination, and majority group more by the cumulative exposure to socioeconomic disadvantage. Further, smaller total brain volumes of majority children partly underlie less optimal cognitive functions due to poverty. If replicated with repeated MRI assessments, our findings could provide scientific support for anti-poverty programs aimed to tackle different mechanisms and possibly distinct vulnerabilities across majority and minority groups.

METHODS

Participants

Our study was embedded in the Generation R Study, a prospective population-based birth cohort in Rotterdam, the Netherlands. Pregnant women with an expected delivery date from April 2002 to January 2006 were invited. The study was described in detail elsewhere (Jaddoe et al., 2012) and approved by the Medical Ethics Committee of the Erasmus Medical Center. Written informed consent was obtained from all adult participants.

In total, 5311 pregnant women provided data on standardized household income (i.e. data on household income and family size) in pregnancy. Of these, those without data on standardized household income in childhood (n = 110), and children without brain magnetic resonance imaging (MRI) data (n = 2413) were excluded. Further, 500 children were excluded due to: poor MRI data quality (n = 414), having braces (n = 58), different T1 acquisition (n = 19), or incidental findings (n = 9). Siblings were randomly excluded (n = 122) to keep only one child from each household. A total of 2166 children were included in our analytical sample (Figure 1).

Poverty

We defined poverty as living under the national low-income threshold in the Netherlands (e.g. (*Armoedebericht*)). Low-income threshold was set to the welfare benefit level of a one-person household in 1979, adjusted for purchasing power taking into account the price change over time (*Armoedebericht*, 2001). An equivalence factor, which was determined based on the number of adults and children and the age of children of household, was used to make incomes of different types of households mutually comparable (Siermann et al., 2004). For example, the low-income threshold for single person was 9,435 euros per year, while the threshold for household of married couple with two children was 15,543 euros and that for single parent with two children was 14,164 euros in the year 2000 (*Armoedebericht*, 2001). The number of adults and children living of the same income and the monthly disposable household income were reported at 30 weeks of pregnancy and twice during childhood, when children were 3 and 5 years old. The latter assessments were combined, as income stability is high during early childhood (Hair et al., 2015). Missing values in family size were imputed using available data at other time points. Income data was originally collected in categories and recoded as numeric variables by taking the midpoint of each bin. The top category for each income assessment was filled with estimates obtained with the Pareto Curve (Parker & Fenwick, 1983). The standardized household income was calculated from the family size and the household income. By comparing to the national low-income threshold, children's poverty exposure was categorized as "never" or "ever" depending on whether

their family experienced poverty at any assessment period. The "ever poverty" exposure was further categorized as "poverty in pregnancy only", "poverty in childhood only", or "chronic poverty (poverty in both pregnancy and childhood)".

Brain imaging

Neuroimaging data were collected with structural acquisition and processing protocols, as described previously (White et al., 2018). Brain magnetic resonance imaging (MRI) was conducted with a 3.0 Tesla MRI scanner (MR750w, General Electric, Milwaukee, WI, USA) using an 8-channel head coil. High-resolution T1-weighted structural MRI data were acquired with a 3D coronal inversion recovery fast spoiled gradient recalled sequence (repetition time = 8.77ms, echo time = 3.4ms, inversion time = 600ms, flip angle = 10°, acquisition matrix = 220*220, field of view = 220mm * 220mm, slice thickness = 1.0mm, number of slices = 230, ARC acceleration factor = 2). Details could be found elsewhere (White et al., 2018). Data were processed using the FreeSurfer version 6.0 analysis suite (Fischl, 2012). Images were processed for cortical reconstruction and volumetric segmentation to obtain the volumes of regions of interests, i.e. total brain, cortical gray matter, cerebral white matter, hippocampus, and amygdala (Muetzel et al., 2019). Data quality of the MRI scans was rated systematically by comparing the white and pial surface representations against the brain image at several slices, and brain scans deemed as unsuitable for analyses were excluded (Figure 1) (Muetzel et al., 2019; White et al., 2018). We compared children participating in the MRI assessment and those not included due to poor imaging quality data (Supplementary Table 1), and found no substantial differences between these groups.

Covariates

Maternal education, maternal and paternal psychiatric symptoms, and maternal national origin were assessed at pregnancy. Maternal education was categorized as "low" to "high" based on the Dutch standard classification of education (Schaart et al., 2008) in accordance with the International Standard Classification of Education (ISCED) (*International Standard Classification of Education (ISCED)*, 1976). Psychiatric symptoms were evaluated using the Brief Symptom Inventory, a validated self-report questionnaire (De Beurs, 2004; Derogatis, 1993) and the Global Severity Index based on 53 items was used for analysis. Maternal national origin was divided into "Dutch", "Non-Dutch Western", and "Non-Western" based on the birthplace of the parents of the adult respondents, following the definitions used by the Statistics Netherlands (*Statistical Yearbook of the Netherlands 2004*, 2004) to define majority and minority statuses. Non-Dutch Western included European, American, Indonesian, Japanese and Oceanian. Non-Western included Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, African, middle and south American and Asian (except for Indonesian and Japanese). Maternal intelligence quo-

tient (IQ) was assessed when children were 5 to 7 years old as a non-verbal intelligence with a computerized version of the Ravens Advanced Progressive Matrices Test, set 1 (Chiesi et al., 2012). Child height was measured at the Research center approximately 1-2 months prior to brain measurement using standardized procedures (Kooijman et al., 2016).

Ethnic discrimination score was calculated based on maternal report on experiences living in a multicultural society assessed in the third trimester of pregnancy (Qureshi et al., 2021). Briefly, mothers scored from 0 to 4 for a total of 4 questions related to negative intergroup experiences ("I have been taunted or insulted due to my ethnic background", "I have been threatened or attacked due to my ethnic background", "I do not feel accepted by Dutch people", and "I feel that Dutch people have something against me"), thus overall score ranged from 0 to 16. Higher score indicates experience of higher levels of ethnic discrimination.

School performance was measured with the CITO test, a mandatory academic test conducted in the final grade of primary school (children are on average 11 to 12 years old), most frequently used to guide the choice for secondary education. The test was developed by the Central Institute for Test Development (Centraal Instituut voor Test Ontwikkeling, CITO) (van der Lubbe, 2018). Test score was standardized and ranged from 500 to 550, with higher score indicating higher levels of school performance.

Non-response

There were some differences in socioeconomic status between children with complete data for poverty status and brain MRI (i.e. included sample) and those with no available data for income during childhood and brain MRI (i.e. excluded sample) (Supplementary Table 1). Briefly, children in poor households were less likely to participate in the follow-up assessments than children in nonpoor households. Also, childhood income and MRI data were more often available among higher educated mothers.

Missing covariate data (maximum missingness of 27.2% in paternal psychiatric symptoms) were imputed with multiple imputation by chained equations using predictive mean matching from the "mice" package (Buuren & Groothuis-Oudshoorn, 2010) in R including exposure (household income) and outcomes (brain morphological measures) as well as covariates as predictors, and 30 imputed datasets were generated.

Analyses

First, linear regression analyses were conducted to elucidate the association between exposure to poverty (never (reference) vs ever exposed to poverty) and brain volumes (total brain, cortical gray matter, cerebral white matter, hippocampus, and amygdala). Analyses were also performed by timing of exposure (never being poor vs poor in pregnancy only, poor in childhood only and chronically poor). In model 1, child sex, child

age at brain measurement, and maternal national origin were included as covariates. In a second model, we further adjusted for maternal education and maternal IQ to control for the confounding effect of social and cognitive factors that are moderately-to-highly heritable, and likely antecedents of poverty. Lastly, we ran a model additionally adjusted for maternal and paternal psychiatric symptoms, which can be conceptualized both as antecedents and consequences of poverty. These variables were seen as potential confounders, hence included in this model. Intracranial volume was included in all models of hippocampus and amygdala volumes. Brain outcomes were standardized to allow comparison across metrics. Post-hoc analysis examined the difference in the associations between exposure to poverty and amygdala volume according to timing of exposure by calculating the 84% confidence interval (CI) for each coefficient (Julious, 2004). We combined poverty at pregnancy only and chronic poverty as "any poverty in pregnancy" and compared with poverty in childhood only (i.e. no poverty exposure in pregnancy) for comparison of associations.

The analysis of the association between poverty exposure (never vs ever) and brain volumes was repeated in Dutch and non-Western groups to examine effect modification by majority and minority groups. A formal interaction test was also performed by the addition of a multiplicative term (poverty * ethnicity). We did not further analyze the non-Dutch Western group since too few were exposed to poverty to provide reliable estimates (total: n = 271; ever being poor: n = 30). In each stratum, associations by timing of exposure were also analyzed. Analyses in the non-Western group were additionally adjusted for detailed maternal national origin.

We further conducted the mediation analysis to examine whether total brain volumes accounted for the association between ever being exposed to poverty and cognitive functions in Dutch majority children. To perform mediation analysis, we imputed missing data including exposure, outcomes, and covariates of the mediation analysis model with expectation-maximization algorithm with R package "Amelia II" (Honaker et al., 2011), which enabled us to obtain 1 imputed dataset that provides precise estimates as multiple imputation does. Thus, mediation analysis was conducted on this 1 acquired dataset using R package "mediation" (Tingley et al., 2014). Mediation model included the same covariates as main analysis model 3, i.e. child sex, child age at brain measurement, maternal national origin, maternal education, maternal IQ, and maternal and paternal psychiatric symptoms. In the outcome model, child age at CITO measurement was additionally adjusted. Averaged causal mediation effect, averaged direct effect, total effect, and proportion of mediated were calculated using the nonparametric bootstrap for variance estimation with 1000 simulations. All analyses were performed with R version 3.6.3 (R Core Team, 2020).

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AUTHOR CONTRIBUTIONS

TW, PJ, and HT conducted the surveys. YK, ACH and HT conceived the study and wrote the first draft of manuscript. HT supervised the statistical analysis. TH, RL, TW, PJ, TF, and HT gave comments on the analysis and manuscript. YK, ACH, and HT finalized the manuscript.

CONFLICT OF INTERESTS

There is no conflict of interests to declare.

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TABLE OF CONTENTS OF SUPPLEMENTARY MATERIALS

Supplementary Table 1. Sample characteristics differences between participants in the analytical sample and those who were lost to follow-up

Characteristics		With poverty data in pregnancy (N = 5311)		With complete poverty and brain MRI data (N = 2788)		With complete poverty and good quality brain MRI data (N = 2288)	
Income in pregnancy, N, %	Available	5311	100.0	2788	100.0	2288	100.0
	Never	4373	82.3	2362	84.7	1950	85.2
	Ever	938	17.7	426	15.3	338	14.8
	Missing	0	0.0	0	0.0	0	0.0
Income at 3yo, N, %	Available	3889	73.2	2239	80.3	1837	80.3
	Never	3335	85.8	1961	87.6	1624	88.4
	Ever	554	14.2	278	12.4	213	11.6
	Missing	1422	26.8	549	19.7	451	19.7
Income at 5yo, N, %	Available	4652	87.6	2589	92.9	2134	93.3
	Never	4049	87.0	2290	88.5	1895	88.8
	Ever	603	13.0	299	11.5	239	11.2
	Missing	659	12.4	199	7.1	154	6.7
Child sex, N, %	Available	5311	100.0	2788	100.0	2288	100.0
	Male	2665	50.2	1367	49.0	1126	49.2
	Female	2646	49.8	1421	51.0	1162	50.8
	Missing	0	0.0	0	0.0	0	0.0
Child age at MRI measurement (years), mean, SD		10.1	0.6	10.1	0.6	10.1	0.6
	Missing	2310	43.5	0	0.0	0	0.0
Maternal national origins, N, %	Available	5304	99.9	2787	100.0	2288	100.0
	Dutch	3243	61.1	1743	62.5	1451	63.4
	Non-Dutch Western	655	12.3	353	12.7	289	12.6
	Non-Western	1406	26.5	691	24.8	548	24.0
	Missing	7	0.1	1	0.0	0	0.0
Maternal IQ, mean, SD		98.2	14.3	99.3	13.9	99.5	13.7
	Missing	847	15.9	191	6.9	146	6.4
Maternal education at pregnancy, N, %	Available	5165	97.3	2720	97.6	2234	97.6

Supplementary Table 1. Sample characteristics differences between participants in the analytical sample and those who were lost to follow-up (*continued*)

Characteristics			With poverty data in pregnancy (N = 5311)		With complete poverty and brain MRI data (N = 2788)		With complete poverty and good quality brain MRI data (N = 2288)	
		High	1572	30.4	886	32.6	743	33.3
		Mid-high	1239	24.0	692	25.4	580	26.0
		Mid-low	1481	28.7	768	28.2	619	27.7
		Low	873	16.9	374	13.8	292	13.1
	Missing		146	2.7	68	2.4	54	2.4
Parental psychiatric symptoms at pregnancy, median, interquartile range	Mother		0.15	0.3	0.14	0.2	0.14	0.2
	Missing		696	13.1	301	10.8	252	11.0
	Father		0.06	0.2	0.06	0.1	0.06	0.1
	Missing		1575	29.7	784	28.1	619	27.1
Maternal age at child birth, mean, SD			31.5	4.7	31.9	4.5	31.8	4.4
	Missing		0	0.0	0	0.0	0	0.0

Supplementary Table 2. Sample characteristics by maternal ethnicity (N = 1895)

Characteristics		Dutch N = 1365		Non-Western N = 530	
Child sex, N, %	Male	679	49.7	254	47.9
	Female	686	50.3	276	52.1
Child age at MRI measurement (years), mean, SD		10.1	0.6	10.1	0.6
Maternal education at pregnancy, N, %	High	533	39.1	57	10.8
	Mid-high	400	29.3	81	15.3
	Mid-low	329	24.1	214	40.3
	Low	103	7.6	178	33.5
Maternal IQ, mean, SD [a]		102.0	12.3	90.5	14.6
Parental psychiatric symptoms at pregnancy, median, interquartile range	Mother	0.12	0.2	0.23	0.5
	Father	0.06	0.1	0.10	0.2
Poverty experience, N, %	Never	1250	91.6	233	44.0
	Ever	115	8.4	297	56.0
	Pregnancy	22	1.6	77	14.5
	Early childhood	58	4.2	52	9.8
	Chronic	35	2.6	168	31.7
Ethnic discrimination, mean, SD		NA	NA	3.21	3.4

The data was combined across imputed datasets.
Non-Western includes Cape Verdean, Moroccan, Dutch Antilles, Surinamese, Turkish, African, American non-Western, Asian non-Western.
a: IQ difference between mothers of Dutch and non-Western origins may reflect lower access to educational opportunities by mothers of the 1st generation of immigrants.

Supplementary Table 3. The association between poverty and brain morphology with interaction for child sex (N = 2166)

	Interaction term		
	B	95%CI	P-value
Total brain volume	0.02	-0.15 to 0.20	0.78
Cortical gray matter volume	-0.02	-0.19 to 0.16	0.85
Cerebral white matter volume	0.04	-0.14 to 0.21	0.70
Mean hippocampus volume	0.06	-0.10 to 0.22	0.47
Mean amygdala volume	-0.06	-0.22 to 0.11	0.49

Model adjusted for child age at brain measurement, maternal ethnicity, maternal education at pregnancy, maternal IQ, maternal and paternal psychiatric symptoms at pregnancy.
Model for subcortical structures (i.e., hippocampus and amygdala volumes) further adjusted for total intracranial volume.
All brain measures of outcome are standardized.
Interaction term was made between poverty status (never vs ever) and child sex (boy vs girl).

Supplementary Table 4. The association between poverty and brain morphology with interaction for maternal ethnicity (N = 1895)

	Interaction term		
	B	95%CI	P-value
Total brain volume	0.22	-0.001 to 0.43	0.05
Cortical gray matter volume	0.17	-0.05 to 0.39	0.14
Cerebral white matter volume	0.24	0.01 to 0.46	0.04
Mean hippocampus volume	-0.13	-0.34 to 0.07	0.20
Mean amygdala volume	-0.07	-0.28 to 0.13	0.48

Model adjusted for child age at brain measurement, child sex, maternal education at pregnancy, maternal IQ, maternal and paternal psychiatric symptoms at pregnancy.
Model for subcortical structures (i.e., hippocampus and amygdala volume) further adjusted for total intracranial volume.
All brain measures of outcome are standardized.
Interaction term was made between poverty status (never vs ever) and maternal ethnicity (Dutch vs non-Western).

Section B

Protective factors, adversity and brain morphology

7

Observed infant-parent attachment and brain morphology in middle childhood- A population-based study

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ABSTRACT

Poor quality of the early infant-parent bond predicts later child problems. Infant-parent attachment has been suggested to influence brain development, but this association has hardly been examined. In adults, larger amygdala volumes have been described in relation to early attachment disorganization; neuroimaging studies of attachment in children, however, are lacking.

We examined the association between infant-parent attachment and brain morphology in 551 children from a population-based cohort in the Netherlands. Infant-parent attachment was observed with the Strange-Situation Procedure at age 14 months and different brain measures were collected with magnetic resonance imaging at mean age 10 years.

Children with disorganized infant attachment had larger hippocampal volumes than those with organized attachment patterns. This finding was robust to the adjustment for confounders and consistent across hemispheres. The association was not explained by cognitive or emotional and behavioral problems. Disorganized attachment did not predict any other difference in brain morphology. Moreover, children with insecure organized infant attachment patterns did not differ from those who were securely attached in any brain outcome.

Causality cannot be inferred, but our findings in this large population-based study provide novel evidence for a long-term association between the quality of infant-parent attachment and specific brain differences in childhood.

INTRODUCTION

Infants have an innate tendency to seek parental protective proximity in stressful situations and this behavior is fostered by consistently available parents (Van IJzendoorn et al., 1999). If the caregiver is not consistently responsive, infants form an insecure, but organized attachment pattern (i.e. avoidant or resistant)(Van IJzendoorn et al., 1999). Some infants, however, develop a disorganized attachment, another typical variation of infant attachment. Infants with disorganized attachment display contradictory or stereotypical behavior when exposed to stress (Granqvist et al., 2017). This attachment pattern is considered to elevate the risk for later dissociative behavior and externalizing behavioral problems (Van IJzendoorn et al., 1999).

Infant attachment insecurity (i.e. avoidant, resistant or disorganized) has been hypothesized to influence brain development; in particular, amygdala and hippocampal morphology (Moutsiana et al., 2015). Although these limbic structures start developing in fetal life, a period of rapid growth occurs during infancy (Lupien et al., 2009). In addition, the development of the amygdala and the hippocampus is stress-sensitive. The stress hormone cortisol has a documented effect on the maturation and remodeling of axons and dendrites (Rinne-Albers et al., 2013), and the amygdala and the hippocampus have a high density of cortisol receptors, implying developmental vulnerability in conditions of sustained stress (Lupien et al., 2009). Animal research has shown that early psychosocial deprivation and poor caregiving conditions affect hypothalamic-pituitary-adrenal (HPA) axis functioning and cortisol production (Lupien et al., 2009). Moreover, an effect of early life stress on hippocampus-dependent memory functioning (Bonapersona et al., 2019) and amygdala and hippocampus morphology has been described (Bath et al., 2016; Coplan et al., 2014).

The association between highly adverse early caregiving conditions and brain morphology has repeatedly been examined in humans. Most studies in adults describe that the exposure to early life adversity is related to smaller hippocampal volumes (see for a meta-analysis, (Riem et al., 2015)), but not to amygdala volumetric differences (see for a meta-analysis, (Calem et al., 2017)). In children, however, the evidence is less consistent. Some studies reported no difference in the amygdala or hippocampal volumes between children with a history of maltreatment and those reporting no maltreatment (De Brito et al., 2013; Riem et al., 2015). Moreover, in the studies where differences were observed in the volume of these limbic structures, the direction of effect varied. McLaughlin et al. (2016) showed that the exposure to maltreatment was related to smaller amygdala and hippocampal volumes in a sample of 60 children aged 6 to 18 years old. Similar findings were described in relation to abuse and early life adversity (Brooks et al., 2014; Hanson et al., 2015). In contrast, Tupler and De Bellis (2006) observed larger hippocampal volumes in 4 to 17-year-old children with maltreatment-related PTSD, and Tottenham et al.(2010)

found larger amygdala volumes in children after prolonged institutional rearing. Many factors may contribute to the heterogeneity across the studies on child maltreatment and limbic morphology, including the small size of most samples, unmeasured confounding by comorbid psychiatric disorders and additional stressors; and the variation in exposures and timing (Bick & Nelson, 2016). The type of adversity, the timing of the exposure occurrence and of the brain morphology measurement play a particularly important role. First, studies examining multiple types of adversity at the same time (such as physical abuse, sexual abuse and neglect) or traumatic events of great severity generally described smaller volumes of the amygdala and the hippocampus (Brooks et al., 2014; McLaughlin et al., 2016). Second, the timing of occurrence and the duration of the adversity are crucial because adversity occurring during different stages of brain development may affect it differently. In fact, Tottenham et al.(2010) found larger amygdala volumes only in children exposed to a longer period of institutional rearing, compared to those who were adopted early and those never institutionalized. Further, the child age at the brain morphology assessment may also affect results. The amygdala and hippocampus have non-linear developmental trajectories during childhood (Uematsu et al., 2012). Thus, the normal development may mask or change the association between adversity and the limbic volumes. Whittle et al. (2013) described seemingly contrasting findings in a longitudinal study that point to this explanation. Higher levels of childhood maltreatment were related to larger hippocampal volumes in early adolescence, but to a decrease in the normal hippocampal growth from early to mid-adolescence (Whittle et al., 2013).

The association between the early child-parent relationship and brain morphology has also been examined in the general population. Although these studies are less confounded by factors that affect clinical samples, similarly inconsistent findings have been reported. Contrasting results can likely be attributed to the small sample sizes, differences in the sample characteristics, and the variation in the age of assessments. Most of these studies have focused on parental behavior, such as sensitivity or support. Two studies examined maternal sensitivity, observed during a non-stressful situation, in relation to brain structure in infancy. Rifkin-Graboi et al.(2015) described in 20 infant-mother dyads that reduced maternal sensitivity was related to larger hippocampal volumes, and Sethna et al.(2017) found an association between reduced sensitivity and smaller subcortical grey matter volumes (including the caudate, putamen, globus pallidus and thalamus) in a sample of 39 infants. A relation of early maternal sensitivity with brain volumes at later ages was documented by Bernier et al.(2019), who described that two dimensions of maternal sensitivity predicted smaller amygdala and hippocampal volumes in 33 10-year-old children. In contrast, higher levels of early parental sensitivity were not associated with the volume of these limbic structures, but predicted larger total brain and gray matter volumes in a subsample of 7-8-year-old children from the pres-

ent cohort (N=191)(Kok et al., 2015). Two studies examined other measures of maternal behavior and found similarly heterogeneous results. Luby et al.(2012) assessed maternal support in early childhood and found a positive relation with hippocampal volumes at ages 7-13 years (N=92), whereas Rao et al.(2010) described that children receiving more parental nurturance, observed at age 4 years, had smaller hippocampal volumes at age 14 years (N=49).

In contrast to this diverse literature documenting the neural correlates of early parental behavior in children from the general population, remarkably little is known regarding the association of infant-parent attachment, as a direct indicator of the child-parent relationship, and brain morphology. A few studies investigated this association in adults and only one focused on children. Leblanc et al.(2017) reported no association between early attachment security and amygdala volume in 33 10-11 year-old children, but larger grey matter volumes in regions of the temporal, frontal and parietal lobes in children who were securely attached in infancy. Moutsiana et al.(2015) examined 59 infant-parent dyads and observed that the insecurely attached infants had larger amygdala volumes as 22-year-old adults than those previously securely attached; no difference in hippocampal volumes was found. Lyons-Ruth et al. found in a sample of 18 29-year old adults from impoverished, highly-stressed families that the 12 adults with disorganized attachment at 18 months had greater amygdala volumes (2016).

Early socioemotional deprivation and childhood trauma have also been described to influence the maturation of white matter microstructure in children (Daniels et al., 2013; Siehl et al., 2018). However, few studies have examined the association between infant-parent attachment and the white matter microstructure in the general population. A positive correlation between attachment security and fractional anisotropy of several tracts including the uncinate fasciculus and the hippocampal part of the cingulum was reported in an adult sample (Serra et al., 2015). Yet, in this study childhood attachment security was assessed with a retrospective self-reported measure, which could influence accuracy. Only one study has prospectively examined whether attachment security is related to white matter microstructure in children, and results were in the opposite direction compared to the adult sample. Dégeilh et al.(2019) found that lower attachment security at age 2 years predicted higher fractional anisotropy and lower mean diffusivity in a number of tracts at age 10 years, including the cingulum bundle. Given the scarcity and methodological limitations of the literature on the association between early attachment and later white matter microstructure, previous studies must be viewed as preliminary, thus precluding a hypothesis-driven approach when examining child white matter microstructure in relation to the early infant-parent bond.

We evaluated the association between infant attachment and brain morphology in middle childhood using a population-based sample (N=551). We examined the hippocampal and amygdala volumes as regions of interest, based on theoretical and

biological evidence for an association between adverse early caregiving experiences and the development of limbic structures. We hypothesized that insecure and especially disorganized patterns of infant-parent attachment are associated with differences in hippocampal and amygdala volumes in children. We additionally included the thalamus volume as a negative control sub-cortical structure, in which no effects were expected a-priori. Considering the scarcity of the existing literature regarding the association between early caregiving and brain regions other than the amygdala and hippocampus, we examined the relation between infant attachment and global brain structural metrics, vertex-wise cortical volume, and global white matter microstructural metrics with an exploratory approach. As we were particularly interested in the limbic structures, we additionally explored the association of infant attachment with white matter tracts related to the limbic system.

METHODS AND MATERIALS

Settings and population

This study was embedded in the Generation R Study, an ongoing population-based cohort in Rotterdam, the Netherlands (Kooijman et al., 2016). The Generation R Study follows children of mothers with a delivery date from April 2002 to January 2006 (61% response at baseline). From the children of the 9778 mothers enrolled in the study, a subsample with Dutch background (i.e. children whose parents and grandparents were born in the Netherlands) was randomly selected for detailed assessments, such as behavioral observations. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, and informed consent was obtained from all participating parents and children.

Among the 1106 infant-parent dyads participating in the postnatal phase of this subgroup, 882 visited the research center at age 14 months, during which infant-parent attachment was assessed (Tharner et al., 2011). When one parent participated in the assessment of attachment with two children, we randomly excluded one (n=24). We also excluded 29 children for whom attachment quality could not be coded because of technical or procedural problems. Brain MRI scans were obtained when children were 10 years old. Of the 829 children with attachment data, 588 (71%) had brain-imaging data. Children with poor image quality of the structural MRI data were excluded from the structural MRI analyses (n=86), as were children with major incidental findings (n=2). Similarly, 85 children with non-usable DTI data and 1 child with a major incidental finding were excluded from the DTI analyses. In total, 551 children were included in one or more analyses (500 with structural MRI and 502 with DTI data; Supplementary Figure 1).

Measures

Attachment Assessment

Infant-parent attachment was assessed in relation to the primary caregiver with the Strange Situation Procedure when infants were 14.6 (SD=0.9) months old (Tharner et al., 2011). This validated procedure is designed to evoke mild stress in the infant and trigger attachment behavior (Ainsworth, 1978). It consists of eight 3 minute-episodes in which the parent leaves the infant in a room twice; first with a female stranger, and later leaving the infant alone. After each separation, the parent reenters the room and the behavior of the child during these reunion episodes is observed. Due to limited time the pre-separation episodes were slightly shortened without impact on the validity of the measures (Tharner et al., 2011). Two reliable raters, trained and supervised, coded the attachment behavior from DVD-recordings, according to the Ainsworth et al.(1978) and Main and Solomon (1990) coding systems. Inter-rater agreement was based on 70 cases independently coded by both raters. The inter-rater agreement on the ABCD attachment classification was 77% (kappa=0.63), and the inter-rater agreement on disorganized *versus* non-disorganized attachment was 87% (kappa =0.64)(Tharner et al., 2011). As previously described (Tharner et al., 2012), the distributions of attachment security and disorganization in our study cohort did not differ from those reported in a meta-analysis of normative non-US western samples.

Brain imaging

Acquisition:

Magnetic resonance imaging was performed when children where 9 to 11 years old. Children were familiarized with the scanning environment in a mock scanning session, prior to the actual scanning session. Brain images were acquired on a 3 Tesla scanner (General Electric MR750w, Milwaukee, WI, USA) with an eight-channel head coil for signal reception. Details of the images acquisition are provided elsewhere (White et al., 2018). High-resolution T1-weighted images were obtained with an inversion recovery fast-spoiled gradient recalled sequence (sequence parameters: TR =8.77 ms, TE=3.4 ms, TI=600 ms, Flip Angle=10°, Field of View (FOV)=220x220 mm, Acquisition Matrix= 220x220, slice thickness= 1 mm, number of slices=230, Parallel Imaging Factor=2). The diffusion weighted images were collected with an axial spin echo, echo-planar imaging sequence with 3 volumes with b=0 s/mm^2 (no diffusion weighting) and 35 diffusion-weighted images (sequence parameters: TR =12,500 ms, TE =72.8 ms, FOV =240x240 mm, Acquisition Matrix =120x120, slice thickness =2 mm, number of slices =65, Asset Acceleration Factor =2, b = 900 s/mm^2).

Image Processing:

Cortical reconstruction and volumetric segmentation were conducted with the FreeSurfer image suite version 6.0 (http://surfer.nmr.mgh.harvard.edu/). In brief, removal of non-brain tissue, voxel intensity normalization, segmentation of subcortical structures, cortical reconstruction and definition of anatomic metrics were performed. FreeSurfer morphometric processes have shown good test-retest reliability (Han et al., 2006). The cortical volume-based map for each participant was smoothed with a 10mm full width, half-maximum Gaussian kernel. The anatomical metrics included in analyses were total brain, total gray matter and cortical white matter volumes, average cortical thickness, and the mean volume (averaged over both hemispheres) of the amygdala, hippocampus and thalamus, and vertex-wise cortical volume.

The diffusion tensor imaging (DTI) data was processed with the FMRIB Software Library (FSL)(Jenkinson et al., 2012), and the Camino diffusion MRI toolkit (Cook et al., 2006). Non-brain tissue was removed and images were corrected for motion and eddy-current artifacts. The resulting transformation matrices were used to rotate the gradient direction table to account for rotations applied to the data. The diffusion tensor was fit at each voxel, and common scalar metrics (global fractional anisotropy (FA) and mean diffusivity (MD)) were computed. Fully-automated probabilistic tractography was run using a set of predefined seed and target masks, resulting in connectivity distributions for a number of large fiber bundles (de Groot et al., 2015). Mean FA and MD were extracted from each tract, and confirmatory factor analysis was used to generate latent FA and MD measures across 12 tracts which represent global white matter microstructure across the brain (cingulum bundle, corticospinal tract, forceps major, forceps minor, inferior longitudinal fasciculus, superior longitudinal fasciculus and the uncinate fasciculus) (Muetzel et al., 2018). (For more details on the probabilistic tractography, see the Supplementary Materials).

FreeSurfer image reconstructions of the T_1 images were visually inspected for quality and all scans rated as unusable were excluded from statistical analyses (Muetzel et al., 2018). Diffusion image quality was assessed by manual and automated inspection. For more information on the image quality inspection see the Supplementary Materials.

Covariates

Potential confounders were selected *a priori* based on previous research (Lyons-Ruth et al., 2016; Moutsiana et al., 2015; Tharner et al., 2011). These included child sex, birthweight, total intracranial volume, age at the MRI scan, smoking and alcohol use during pregnancy, maternal education, maternal psychiatric symptoms and breastfeeding. Information on child sex and birthweight was obtained from midwives and hospital registries. Total intracranial volume was extracted from the processed structural imaging data. Child age at the MRI scan was based on the date of birth and date of the imaging

data collection. Maternal self-reports of prenatal smoking and alcohol consumption were collected during pregnancy. Maternal education was self-reported in pregnancy and at two postnatal time points and was classified based on the highest completed education into: low (no bachelor), medium (university bachelor) and high education (further education) (Statistics Netherlands, 2005). Maternal psychiatric symptoms, assessed with the Brief Symptom Inventory (Derogatis, 1993), and current breastfeeding practices (exclusive breastfeeding, breast- and bottle-feeding, and bottle-feeding), were reported by mothers when children were 2 months old.

Traumatic life events, child IQ and children's emotional and behavioral problems were included as covariates in sensitivity analyses. The information on traumatic life events was collected with an interview with the caregiver when children were 9 years old (previously described in Dunn et al.(2019)). In this assessment, caregivers were asked to indicate whether the children had experienced one or more of a list of 24 life events. A cumulative score was created by summing the occurrence of the events, with higher values representing more events. Child IQ was assessed in the research center when children were 5 to 7 years old, with a validated Dutch nonverbal intelligence test: Snijders-Oomen Niet-verbale intelligentie test, 2.5-7- revisie (SON-R 2.5-7) (Tellegen et al., 1998). When children were approximately 9 years old, mothers completed the Child Behavioral Checklist (CBCL) for ages 6-18. The CBCL is a standardized, valid instrument that measures behavioral and emotional problems in children (Achenbach & Rescorla, 2001). In our analyses, we included the Total Problems scale.

Statistical Analysis

We examined two main dimensions of infant attachment. First, we compared children with disorganized infant attachment to those with an organized attachment (i.e. secure, resistant or avoidant). Then, we compared children with an insecure organized attachment pattern (i.e. avoidant or resistant) to those securely attached, excluding the children with disorganized attachment. The mean amygdala and hippocampal volumes were our primary outcomes. The volume of the thalamus was included as a control subcortical structure, to test the specificity of effects. Other brain structural measures (i.e. average cortical thickness and total brain, total gray matter and cortical white matter volumes, and vertex-wise cortical volume) and white matter metrics (global FA and MD) were examined in exploratory analyses. All brain measures were standardized to have a mean of zero and a standard deviation of one.

First, we explored the bivariate associations among the main variables in our study using Pearson's and phi correlations. Then, we examined the association between infant-parent attachment and the brain outcomes with multiple linear regression models, adjusted for child sex, child age at MRI scan, maternal education, maternal psychiatric symptoms and alcohol consumption during pregnancy. Total intracranial

volume was included as a covariate in the analyses with specific brain volumetric measures (i.e. amygdala, hippocampus and thalamus volumes) and white matter connectivity measures (Takao et al., 2011). We included in our models the covariates selected based on literature. As the theoretical evidence for a confounding effect of birthweight, breastfeeding, alcohol consumption and smoking during pregnancy is not very strong and can be debated, we tested the change-in-estimate criterion on our hypothesized associations (i.e. disorganized attachment with hippocampal and amygdala volumes) to decide whether to include them as confounders. Of these variables, only alcohol consumption changed the effect estimate in more than 10%, and thus was included as a confounder (Greenland, 1989; Walter & Tiemeier, 2009). We adjusted for confounders in two models. First, we controlled our analyses for child sex and child age at MRI scan (and total intracranial volume in specific analyses) to take into account brain maturation differences and to facilitate comparison with other studies. Second, we further adjusted the analyses for the confounding effect of the modifiable variables prenatal alcohol consumption, maternal education and maternal psychiatric symptoms.

The associations between attachment disorganization and insecurity with cortical volume were examined at each cortical vertex with similarly adjusted models, using the QdecR package version 2.0 (https://github.com/slamballais/QDECR). To account for multiple testing, cortical volume vertex-wise analyses were adjusted using Gaussian Monte Carlo Simulations (Hagler et al., 2006) with a cluster forming threshold (CFT) of p=0.001 (Greve & Fischl, 2018) and a cluster-wise p-value of $p < 0.025$ (Bonferroni-corrected for two hemispheres).

Several sensitivity analyses were conducted. First, we examined the hemisphere-specific associations with the amygdala and hippocampus. Second, to examine the possibility of misclassification, we repeated our analyses excluding the children who had an attachment classification available that was rated as possibly problematic due to minor technical or procedural difficulties (n=26 for structural, n=23 for DTI). Third, we examined if the exclusion of children with minor incidental findings on the brain image such as asymmetric ventricles changed the results (White et al., 2018) (n=30 for structural, n=29 for DTI). Fourth, we excluded infant-father dyads (n=69 for structural, n=73 for DTI). And fifth, we tested the interaction between child sex and attachment security and disorganization on amygdala and hippocampal volumes.

We additionally adjusted our analyses in separate models for child traumatic life events, child IQ score and child emotional and behavioral problems. Disorganized attachment is more common among infants experiencing traumatic life events (such as maltreatment)(Van IJzendoorn et al., 1999), and such events are also related to hippocampal morphology (Tottenham & Sheridan, 2010). Similarly, the quality of attachment and brain development have been related to cognitive and psychological differences (Granqvist et al., 2017; Harris & Corriveau, 2011; Lenroot & Giedd, 2006). As these fac-

tors may confound the association and also represent proxies of the exposure (i.e. traumatic life events and infant attachment) or outcome (child cognition and behavior and child brain), controlling for these factors could represent overadjustment and bias our associations. We included these variables as covariates in sensitivity analyses, with a hypothesis-generating approach, in an attempt to examine whether they explain the associations between infant attachment and child brain morphology.

All analyses were conducted using the R statistical software (version 3.5.1)(R Core Team, 2020). Missing values (maximum percentage: maternal psychopathology=17.2%) were imputed with the Multivariate Imputations by Chained Equations (MICE) package (version 3.3.0) (van Buuren & Groothuis-Oudshoorn, 2011) generating 20 imputed datasets.

Non-response analysis

We compared the children included in our study (n=551) with the children who were lost to follow-up (n=241) using t-tests and Mann-Whitney U tests for continuous and chi-square tests for categorical variables. We found no difference in child birth weight, sex (study sample: 49% girls, lost to follow-up: 50% girls, p= 0.79) or attachment classification (study sample: secure= 51%, avoidant= 12%, resistant= 15%, disorganized=22%; lost to follow-up: secure=51%, avoidant=14%, resistant=17%, disorganized=18%. p =0.59). Similarly, maternal psychopathology (p=0.33) and maternal education (education in study sample: low: 27%, medium: 31%, high: 42%; in lost to follow-up: low: 31%, medium: 32%, high:37%, p=0.42) did not substantially differ between the groups.

RESULTS

The correlations between the main variables are shown in Supplementary Table 1. No strong correlations were observed between the attachment variables and the covariates. In total, 51% of the children had a secure, 15% a resistant, 12% an avoidant and 22% a disorganized attachment pattern. Table 1 presents the baseline characteristics of the sample for organized and disorganized attachment dyads. Of the children with organized attachment, 49% were girls, while this was 46% in the disorganized attachment group. A larger percentage of mothers had a high education in the organized attachment group (44%) compared to those in the disorganized attachment group (33%, p=0.02). No difference was observed between the organized and disorganized attachment groups regarding child age at the MRI scan, birthweight, child IQ score, child behavioral and emotional problems and maternal psychiatric symptoms. Similarly, the main study variables did not differ when comparing secure and insecure dyads (Supplementary Table 2).

Table 1. Sample characteristics by attachment disorganization

	Organized n= 431 mean(SD) or %*	Disorganized n= 120 mean(SD) or %*	p
Child characteristics			
Sex, % girls	49.2	45.8	0.58
Age at the MRI scan, years	10.1 (0.6)	10.2 (0.6)	0.22
Birth weight, grams	3524.3 (534.1)	3515.5 (534.5)	0.87
Age at the Attachment assessment, months	14.6 (0.9)	14.6 (0.8)	0.93
Attachment classification (%)			-
Secure	65.2	0	
Avoidant	16.0	0	
Resistant	18.8	0	
Disorganized	0	100	
Child IQ score	106.9 (13.0)	107.2 (12.8)	0.69
Child Total Problems score, CBCL global scale, median (range)	13.1 (0, 82.7)	14.5 (0, 58)	0.67
Maternal characteristics			
Education, %			0.02
Low	27.9	25.9	
Medium	28.1	40.8	
High	44.0	33.3	
Maternal Psychopathology, BSI score, median (range)	0.1 (0, 2.3)	0.1 (0, 0.7)	0.34

Characteristics of the sample with available information for attachment and brain structural and/or DTI MRI data (n=551). *Otherwise indicated. Groups were compared in the first imputed dataset with independent t-tests and Mann-Whitney U tests for continuous variables and chi-square tests for categorical variables.

Children with a disorganized infant attachment had, on average, 0.17 standard deviation larger amygdala volumes (SE=0.08, p=0.04) and 0.21 standard deviation larger hippocampal volumes (SE=0.08, p=0.02) than children with organized attachment, accounting for total intracranial volume, child sex and age (Table 2)(see also Figure 1). After additional adjustment for prenatal alcohol consumption, maternal education and psychiatric symptoms the association with mean hippocampal volume remained (b=0.21, SE=0.09, p=0.02), but disorganized attachment was not significantly associated with the amygdala volume (b=0.16, SE=0.08, p=0.06) anymore. No association was observed between disorganized attachment and any of the global brain measures, the thalamus, or the DTI metrics. In addition, we explored the association between attachment disorganization and the microstructure of the white matter tracts related to the limbic system, namely the uncinate fasciculus, the cingulum bundle and the parahippocampal part of the cingulum. We observed higher FA in the left uncinate fasciculus in children with disorganized attachment compared to those with an organized attachment pattern (b=0.22, SE=0.11, p =0.04). However, this association did not survive

multiple testing correction (False discovery rate (Benjamini & Hochberg, 1995) for 12 tests: 3 hemisphere-specific white matter tracts with FA and MD). Although the direction of the association is arguably consistent with that of the structural analysis of the hippocampus, this result should be interpreted with caution.

Table 2. Attachment disorganization and brain morphology

		Brain Outcomes					
		Model 1			Model 2		
	n	b	SE	P	b	SE	P
Determinant							
Disorganized Attachment, yes							
Outcome							
Global brain measures							
Total brain volume	500	-0.08	0.09	0.37	-0.07	0.09	0.47
Total gray matter volume	500	-0.09	0.09	0.33	-0.06	0.09	0.49
Cortical white matter volume	500	-0.07	0.09	0.43	-0.07	0.10	0.44
Total cortical thickness, average	500	0.16	0.11	0.14	0.16	0.11	0.15
Global fractional anisotropy (DTI)	502	-0.04	0.10	0.71	-0.03	0.11	0.80
Global mean diffusivity (DTI)	502	-0.04	0.10	0.69	-0.04	0.10	0.73
Specific brain volumetric measures							
Amygdala volume, average	500	0.17	0.08	0.04	0.16	0.08	0.06
Left Amygdala	500	0.17	0.09	0.05	0.16	0.09	0.07
Right Amygdala	500	0.15	0.09	0.09	0.13	0.09	0.12
Hippocampus volume, average	500	0.21	0.08	0.02	0.21	0.09	0.02
Left Hippocampus	500	0.18	0.09	0.03	0.18	0.09	0.04
Right Hippocampus	500	0.21	0.09	0.02	0.22	0.09	0.02
Thalamus volume, average	500	0	0.07	0.95	-0.01	0.07	0.90

Model 1 was adjusted for: total ICV (total intracranial volume), child age at brain MRI scan, child sex. Model 2 was additionally adjusted for: maternal education, maternal psychiatric symptoms and alcohol use during pregnancy. Global brain structural measures were not adjusted for total ICV. All outcomes were standardized.

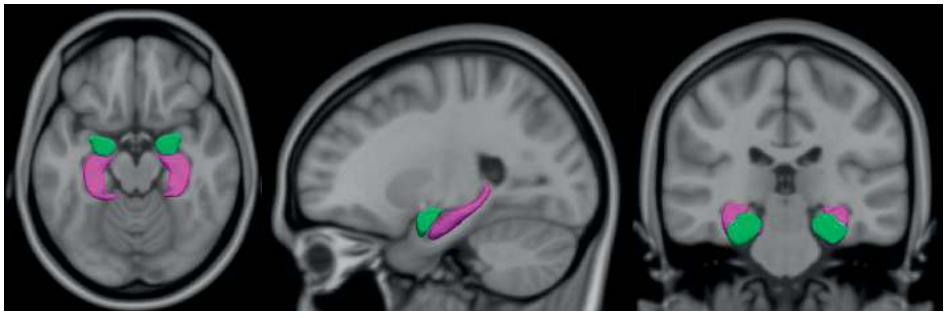

Figure 1. T1-weighted MRI scan (axial, sagittal and coronal view) showing the amygdala (in green) and hippocampus (in purple) segmentation.

Table 3 shows that infants with an organized insecure attachment (i.e. avoidant or resistant) did not differ from those who were securely attached in any of the child brain measures (i.e. mean amygdala, hippocampus and thalamus volumes, average cortical thickness, total brain, total gray matter and cortical white matter volumes and global diffusion metrics).

Table 3. Attachment security and brain morphology

		Brain Outcomes					
		Model 1			Model 2		
	n	b	SE	P	b	SE	P
Determinant							
Insecure Attachment, yes							
Outcome							
Global brain measures							
Total brain volume	390	-0.02	0.09	0.82	-0.02	0.09	0.80
Total gray matter volume	390	0.03	0.09	0.77	0.03	0.09	0.77
Cortical white matter volume	390	-0.07	0.09	0.46	-0.08	0.10	0.42
Total cortical thickness, average	390	-0.02	0.11	0.86	-0.03	0.11	0.77
Global fractional anisotropy (DTI)	392	0.01	0.10	0.90	-0.01	0.10	0.94
Global mean diffusivity (DTI)	392	-0.14	0.10	0.16	-0.11	0.10	0.27
Specific brain volumetric measures							
Amygdala volume, average	390	-0.01	0.08	0.91	-0.03	0.08	0.75
Hippocampus volume, average	390	-0.05	0.08	0.53	-0.05	0.09	0.60
Thalamus volume, average	390	0.07	0.07	0.31	0.08	0.07	0.27

Children with insecure organized attachment (avoidant or resistant attachment) were compared to children with secure attachment, excluding the children with disorganized attachment. Model 1 was adjusted for: total ICV (total intracranial volume), child age at brain MRI scan, child sex. Model 2 was additionally adjusted for: maternal education, maternal psychiatric symptoms and alcohol use during pregnancy. Global brain structural measures were not adjusted for total ICV. All outcomes were standardized.

Whole-brain exploratory analyses were performed to examine the associations of disorganized and insecure infant attachment with vertex-wise cortical volume. No associations were observed after adjusting for multiple testing.

Sensitivity analyses

The positive association of disorganized infant attachment with hippocampal volume was observed consistently in both hemispheres (adjusted left: b=0.18, SE=0.09, p=0.04, adjusted right: b=0.22, SE=0.09, p=0.02). After excluding cases with technical or procedural difficulties in the attachment assessment, disorganization of attachment was still related to larger hippocampal volumes (b=0.22, SE=0.09, p=0.01). Similar results were also obtained after the exclusion of children who had minor incidental findings on MRI;

the difference in hippocampal volume between children with and without disorganized infant attachment was, if anything, larger (b=0.23, SE=0.09, *p*=0.01). The exclusion of infant-father dyads did not meaningfully change the results (disorganized attachment and hippocampal volume, adjusted model: b=0.23, SE=0.09, *p*=0.01) (Supplementary Table 3 and 4). No interaction between disorganized infant attachment and child sex was found in the analyses with amygdala and hippocampal volumes.

The number of traumatic life events did not explain the association between disorganized attachment and hippocampal volume. After adjusting our analyses for traumatic life events, the association between disorganized attachment and hippocampal volume remained unchanged (mean hippocampal volume: b=0.21, SE=0.09, *p*=0.02).

We also explored whether the association between disorganized attachment and hippocampal volume was explained by child IQ, assessed with a non-verbal test at 5 to 7 years of age, or by child emotional and behavioral problems, reported by the mothers with the Child Behavioral Checklist (CBCL) at age 9 years. We found no evidence for this explanation; the effect estimate did not change after additional adjustment for child IQ (mean hippocampal volume: b=0.20, SE=0.09, *p*=0.02) nor after adjustment for the total CBCL score (mean hippocampal volume: b=0.21, SE=0.09, *p*=0.02).

DISCUSSION

In this population-based study, infants with disorganized attachment had larger hippocampal volumes in middle childhood. A similar association between disorganized attachment and amygdala volume did not reach significance. Disorganized attachment was not related to any other brain measure. Organized (in-)security of attachment did not predict any difference in specific or global brain measures.

Although often hypothesized based on biological insights, there is surprisingly little epidemiological evidence for the relation between the quality of the infant-parent attachment relationship and the development of limbic structures. Two small studies reported an association between insecure (including disorganized) infant-parent attachment and larger amygdala volume in adulthood (Lyons-Ruth et al., 2016; Moutsiana et al., 2015). In contrast to adult studies, infant attachment security did not predict any difference in the amygdala volume in a small developmental study (Leblanc et al., 2017).

To date, no study has examined the association between disorganized attachment and the limbic structures in childhood; previous studies broadly examined insecure infant-parent attachment, which likely included some infants with disorganized attachment. In contrast to the organized insecure attachment patterns (i.e. resistant and avoidant), disorganization of attachment is considered a major risk factor for later aggressive behavior and psychopathology (Van IJzendoorn et al., 1999). Additionally, most of the

evidence on the neural correlates of the infant-parent relationship in the general population comes from studies of maternal sensitivity and support. Although the assessment of these maternal behaviors gives insight in the quality of the early caregiving, the infant-parent attachment offers a direct perspective on the infant-parent relationship (De Wolff & van IJzendoorn, 1997). Moreover, maternal sensitivity is known to predict the development of insecure attachment (De Wolff & van IJzendoorn, 1997) but it only weakly predicts the attachment disorganization (Van IJzendoorn et al., 1999). Typical antecedents of this attachment pattern are maltreatment and a parent's unresolved loss or trauma (Granqvist et al., 2017). Thus, these issues need to be considered when interpreting our results in the light of findings on other measures of early caregiving. Moreover, studies on maternal sensitivity and the hippocampal volume are not consistent, with some reporting no difference, others a positive association, and some others a relation with a negative direction of effect. Whereas Kok et al.(2015) found no difference in the hippocampal volumes in a subset of the present cohort, Luby et al.(2012) described a positive relation of maternal support and larger hippocampal volumes in a study oversampled for child depression. Rao et al.(2010), in contrast, observed that less parental nurturance at age 4 years was related to larger hippocampal volume in adolescence, using data from a cohort that studies the prenatal use of cocaine.

We observed that disorganized infant-parent attachment is related to larger hippocampal volume in childhood. This finding may seem counterintuitive as larger volumes often indicate better functioning (Tupler & De Bellis, 2006). However, larger hippocampus and amygdala volumes must be understood within the rubric of developmental trajectories. Both structures undergo non-linear volumetric changes during childhood, develop rapidly during infancy and reach a peak volume during preadolescence (9-11 years) (Uematsu et al., 2012). Thus, the age period in which the brain structures are measured can influence the direction and strength of the association as differences may be masked or distorted by the developmental trajectories. Second, the severity of the adversity and additional co-occurring stressors may also influence results (Bick & Nelson, 2016). Children exposed to extreme adverse experiences such as maltreatment and institutional rearing are not only exposed to more severe adversities but also are likely to experience several other stressors, such as poverty and violence. It is possible that these events affect the brain developmental trajectories in a different way (Bick & Nelson, 2016). Finally, it has been suggested that some brain regions can have an initial accelerated development in response to stress, followed by a volumetric reduction when the exposure to the event is sustained (Callaghan & Tottenham, 2016). The larger hippocampal volume observed in children with disorganized infant attachment could reflect an initial response to stress, induced by disruptions in the infant-parent relationship. Disorganization of attachment is an indicator of stressful experiences, where the infant is confronted with a paradox: their caregiver is the source of fright and comfort at

the same time (Granqvist et al., 2017; Van IJzendoorn et al., 1999). As the hippocampus is involved in the stress response and has large quantities of glucocorticoid receptors (Lupien et al., 2009), stress during infancy can influence its development. The exposure to early stress may induce an initial hypertrophy, increase in dendritic arborization and precocious myelination in the hippocampus, which might be followed by a volumetric reduction only if the exposure to stress continues throughout the life course (Tottenham & Sheridan, 2010). Our findings could be explained by an accelerated hippocampal development in response to challenging environmental factors. As suggested by animal and human studies, poor early caregiving may promote a precocious development of neural regions key in memory and emotion regulation (Bath et al., 2016; Thijssen et al., 2017). This accelerated development has been hypothesized to have evolutionary implications, as it may represent a biological strategy developed to increase survival and reproduction in unfavorable conditions (Belsky et al., 1991).

There are also other potential explanations for the relation between infant attachment and hippocampal volume in middle childhood. First, the hypothalamic hormone oxytocin has been shown to promote neurogenesis in the hippocampus (Sánchez-Vidaña et al., 2016) and to be involved in bonding behavior (Galbally et al., 2011). High oxytocin levels are related to a more stimulating and affective parenting behavior (Abraham & Feldman, 2018) and reduce the cortisol response to stress (Ditzen et al., 2009). In adverse early caregiving conditions, the low oxytocin levels may alter the hippocampal maturation. Although taken together these findings suggest a relation between oxytocin and child social and neural development, the possible role of oxytocin is yet to be elucidated (Galbally et al., 2011). Another possibility is that these limbic structural differences reflect a neurobiological predisposition to the formation of a disorganized infant-parent attachment. In fact, parental behavior only partly explains the etiology of a disorganized attachment, suggesting that other factors, such as genetics and biological infant characteristics, could play a role (Tharner et al., 2011). As described by Spangler et al.,(1996) the status of disorganized attachment may be predicted by newborn emotional regulation and orientation to external stimuli, both of which are hippocampal-related tasks (Bird & Burgess, 2008; Immordino-Yang & Singh, 2013). However, most hypotheses trying to explain the association between infant attachment and limbic morphological differences are still highly speculative. First and foremost, these findings need to be replicated in similarly large population-based samples and the direction of the association needs to be examined with repeated MRI assessments.

In our study, the quality of attachment did not relate to differences in global brain volumetric measures, the vertex-wise cortical volume, or a non-limbic subcortical structure. This suggests that the associations pertain to the development of limbic structures, rather than a globally altered neurodevelopment. Also, although the quality of attachment and the hippocampal development are generally related to psychosocial

adversity, child cognition and behavioral problems, the differences in hippocampal volume remained after these factors were accounted for in the analyses. Therefore, our findings appear to be specific for the disorganization of attachment, rather than explained by factors often related to the attachment quality. If replicated, the specificity of this association would underscore the importance of the early infant-parent attachment quality in the normative neurodevelopment of children.

Small effect sizes are expected for studies of parent-child interaction and subcortical brain structures after birth given that the development of subcortical structures, such as the hippocampus, occurs mostly prenatally and during infancy and less during childhood (Lupien et al., 2009). Thus, although we examined a relatively large sample of children, further population-based studies with large samples and repeated MRI and attachment measures are needed to examine the mechanism and direction of the association. Several limitations of our study should be considered. We cannot exclude reverse causality as disorganized attachment may be a marker of infant stress related to hippocampal development. Also, the sample of infant-father dyads in our study was rather small, precluding the evaluation of the specific relation between infant-father attachment and brain development.

In this study, disorganized early-life attachment was related to larger hippocampal volume in middle childhood. Our findings extend the knowledge on the relation between infant-parent attachment and limbic system morphology with evidence for an association between disorganized attachment and a subcortical structure key to emotional and cognitive processing. Causality cannot be inferred, but our results in a large prospective population-based sample suggest that disorganized infant attachment has a long-term relation with child neurological development.

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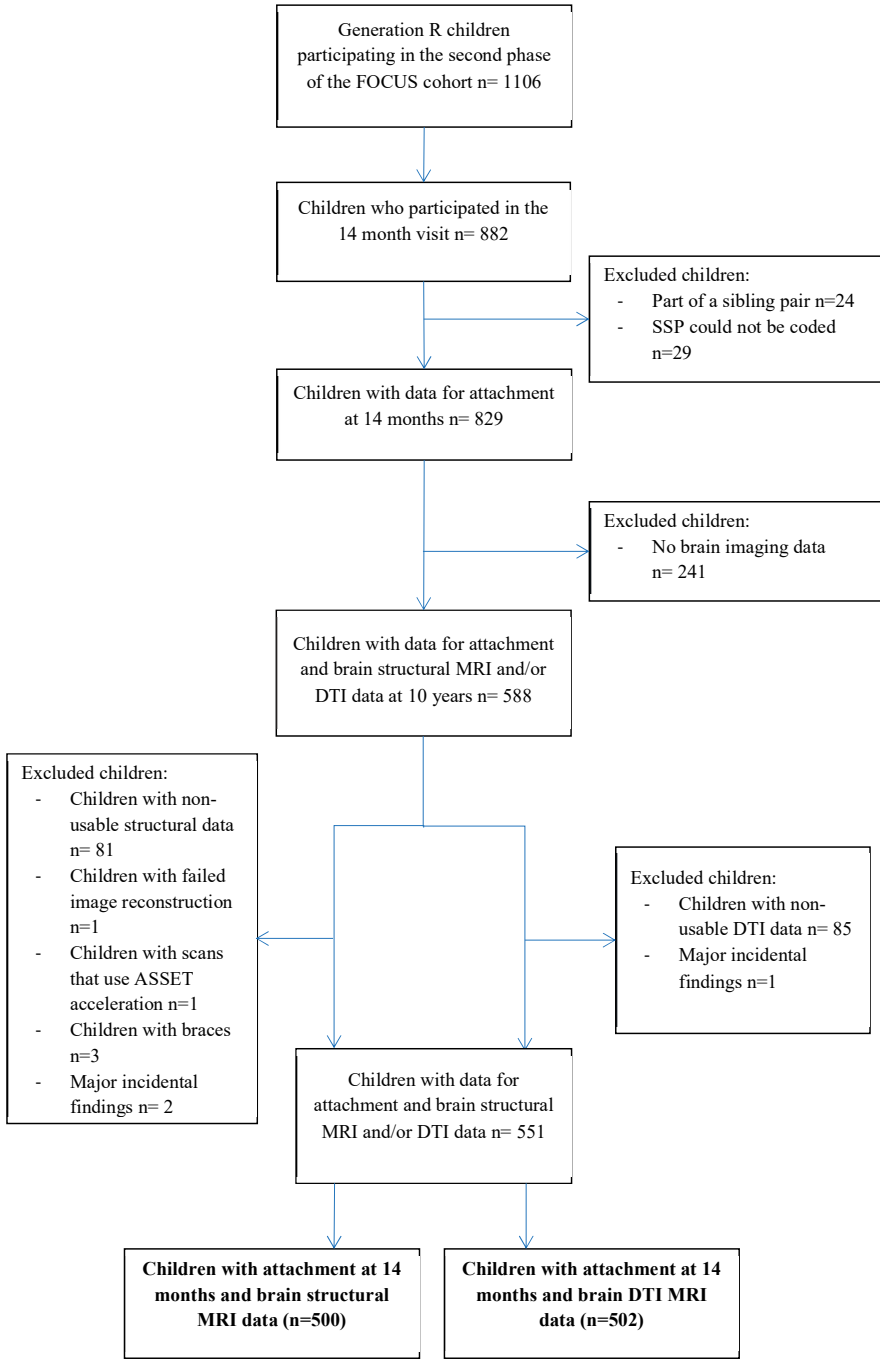
SUPPLEMENTARY MATERIALS



Supplementary Figure 1. Flowchart of sample selection

Supplementary Methods

Fiber tractography:

The diffusion parameters were estimated at each voxel with FSL (BEDPOSTx package) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT/UserGuide#BEDPOSTX), accounting for two fiber orientations. Then, probabilistic fiber tracking was performed to estimate connectivity distributions for a number of large fiber bundles using the FSL Probtrackx module with a set of predefined seed and target masks supplied by the FSL plugin, AutoPtx (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/AutoPtx). Briefly, from each voxel in the seed mask, samples were sent to the target mask (de Groot et al., 2015). The number of samples passing through a given voxel on a successful seed-to-target run were registered, and the resulting distributions were normalized (by the number of total successful seed-to-target attempts) and low-probability voxels were removed. Average DTI scalar metrics (e.g. FA, MD) were computed for each tract, weighted (voxel-wise) by the connectivity distributions.

Image quality assessment

FreeSurfer reconstructions of the T_1 images were visually inspected and rated for accuracy using a five-item scale (unusable, poor, sufficient, good, excellent). All scans that were rated as unusable or poor were excluded from statistical analyses.

Diffusion image quality was assessed by manual and automated inspection. First, the data was automatically inspected with the DTIPrep tool (https://www.nitrc.org/projects/dtiprep/), examining the slice-wise signal variation that is characteristic of artifact in each diffusion-weighted volume. Second, voxel-wise maps of the sum-of-squares error (SSE) of the diffusion tensor fit calculations were evaluated for structured signal indicative of artifacts. Cases with data flagged by the automated or the manual inspection to be of poor quality were excluded from analyses. The quality of probabilistic tractography path reconstructions as well as the nonlinear registration to standard space were examined by visual inspection (Muetzel et al., 2018).

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Supplementary Table 1. Correlations between the main study variables

	n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1.	431	-																	
2.	551	-	-																
3.	551	0.05	0.03	-															
4.	551	0.01	0.05	-0.01	-														
5.	551	-0.07	-0.04	0.1*	0.01	-													
6.	551	-0.06	-0.04	-0.03	0.08*	-0.08	-												
7.	551	0.04	-0.06	0.09*	0.06	0.11**	0.29***	-											
8.	551	-0.01	-0.01	0.01	0	-0.1*	0.17***	0.12**	-										
9.	551	0.09	-0.05	0.01	0.06	0.16***	-0.17***	0.11**	-0.08	-									
10.	551	-0.09	-0.01	0.12**	-0.03	-0.07	0.07	0.09*	0.05	-0.12**	-								
11.	500	0.04	-0.03	0.53***	-0.01	0	0.05	0.13**	0	-0.04	0.26***	-							
12.	500	0	-0.02	0.49***	0.05	0	0	0.08	-0.03	-0.05	0.24***	0.83***	-						
13.	500	0.01	0.03	0.43***	0.02	-0.02	0	0.08	-0.04	-0.03	0.14**	0.7***	0.62***	-					
14.	500	-0.01	0.05	0.39***	0.12**	0.01	0.06	0.03	-0.01	-0.01	0.14**	0.65***	0.59***	0.69***	-				
15.	502	0.01	-0.03	0.04	0.2***	-0.01	0.06	0.05	0.06	-0.01	-0.06	0.19***	0.2***	0.12**	0.17***	-			
16.	502	-0.06	-0.05	0.14**	-0.17***	0.02	0.01	0.05	-0.01	-0.02	0.11*	0.25***	0.26***	0.28***	0.17***	-0.59***	-		
17.	551	-0.09	0.02	0.04	-0.01	0	0.2***	0.1*	0.02	-0.03	0.11**	0.2***	0.16***	0.17***	0.15***	0.04	0.07	-	
18.	551	0.05	0	0.11*	0	0.3***	-0.1*	0	-0.1*	0.07	-0.04	0.01	0.02	0	0.01	0.01	-0.06	-0.08	-
19.	551	0.01	-0.03	-0.01	0.03	0.28***	-0.07	-0.01	-0.03	0.07	-0.03	-0.08	-0.1*	-0.03	-0.05	-0.07	-0.01	-0.06	0.36***

Note: Correlations computed in the first imputed dataset. Bivariate P*earson's correlations* between study variables; for two dichotomous variables, *phi correlation* is used. All brain outcomes are standardized. All dichotomous variables (insecure attachment, disorganized attachment, sex and breastfeeding) are coded as 0/1 variables. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Abbreviations: 1. = Insecure attachment, yes. 2 = Disorganized attachment, yes. 3 = Child sex, male. 4 = Age at the MRI scan, years. 5 = Maternal psychopathology, BSI score. 6 = Maternal education. 7 = Alcohol use in pregnancy. 8 = Exclusive maternal breastfeeding, yes. 9 = Smoking during pregnancy. 10 = Birth weight, grams. 11 = Total gray matter volume. 12 = Cortical white matter volume. 13 = Amygdala volume, average. 14 = Hippocampus volume, average. 15 = Global fractional anisotropy (FA). 16 = Global mean diffusivity (MD). 17 = Child IQ score. 18 = Total emotion and behavior problems score. 19 = Traumatic life events.

Supplementary Table 2. Sample characteristics by attachment security

	Secure n= 281 mean(SD) or %*	Insecure n=150 mean(SD) or %*	p
Child characteristics			
Sex, % girls	50.9	46.0	0.39
Age at the MRI scan, years	10.1 (0.6)	10.1 (0.6)	0.89
Birth weight, grams	3558.7 (494.4)	3459.8 (597.7)	0.08
Age at the Attachment assessment, months	14.6 (0.9)	14.7 (1.0)	0.18
Attachment classification (%)			-
Secure	100	0	
Avoidant	0	46.0	
Resistant	0	54.0	
Disorganized	0	0	
Child IQ score	107.8 (13.0)	105.1 (13.0)	0.06
Child Total Problems score, CBCL global scale, median (range)	13.0 (0, 82.7)	13.8 (0, 70)	0.25
Maternal characteristics			
Education, %			0.13
Low	27.5	28.7	
Medium	25.4	33.3	
High	47.1	38.0	
Maternal Psychopathology, BSI score, median (range)	0.1 (0, 2.3)	0.1 (0, 1.8)	0.09

Characteristics of the sample of children with organized infant attachment with available information for attachment and brain structural and/or DTI MRI data (n=431). *Otherwise indicated. Groups were compared in the first imputed dataset with independent t-tests and Mann-Whitney U tests for continuous variables and chi-square tests for categorical variables.

Supplementary Table 3. Attachment disorganization and brain morphology excluding infant-father dyads

		Brain Outcomes					
		Model 1			Model 2		
	n	b	SE	P	b	SE	P
Determinant							
Disorganized Attachment, yes							
Outcome							
Global brain measures							
Total brain volume	431	-0.09	0.10	0.37	-0.07	0.10	0.49
Total gray matter volume	431	-0.10	0.10	0.31	-0.07	0.10	0.51
Cortical white matter volume	431	-0.08	0.10	0.45	-0.07	0.10	0.47
Total cortical thickness, average	431	0.14	0.11	0.22	0.15	0.11	0.20
Global fractional anisotropy (DTI)	429	-0.04	0.11	0.71	-0.02	0.11	0.86
Global mean diffusivity (DTI)	429	-0.08	0.11	0.48	-0.08	0.11	0.45
Specific brain volumetric measures							
Amygdala volume, average	431	0.14	0.09	0.10	0.14	0.09	0.12
Hippocampus volume, average	431	0.22	0.09	0.01	0.23	0.09	0.01
Thalamus volume, average	431	-0.03	0.08	0.70	-0.04	0.08	0.65

Model 1 was adjusted for: total ICV (total intracranial volume), child age at brain MRI scan, child sex. Model 2 was additionally adjusted for: maternal education, maternal psychiatric symptoms and alcohol use during pregnancy. Global structural brain measures were not adjusted for total ICV. All brain outcomes are standardized.

Supplementary Table 4. Attachment security and brain morphology excluding infant-father dyads

		Brain Outcomes					
		Model 1			Model 2		
	n	b	SE	P	b	SE	P
Determinant							
Insecure Attachment, yes							
Outcome							
Global brain measures							
Total brain volume	328	0.01	0.10	0.94	0	0.10	0.99
Total gray matter volume	328	0.06	0.10	0.56	0.05	0.10	0.60
Cortical white matter volume	328	-0.05	0.10	0.64	-0.06	0.10	0.55
Total cortical thickness, average	328	-0.04	0.11	0.76	-0.03	0.11	0.76
Global fractional anisotropy (DTI)	327	-0.08	0.11	0.45	-0.09	0.11	0.39
Global mean diffusivity (DTI)	327	-0.05	0.11	0.67	-0.03	0.11	0.77
Specific brain volumetric measures							
Amygdala volume, average	328	-0.03	0.09	0.75	-0.03	0.09	0.72
Hippocampus volume, average	328	-0.09	0.09	0.31	-0.08	0.09	0.41
Thalamus volume, average	328	0.06	0.08	0.47	0.06	0.08	0.42

Children with insecure organized attachment (avoidant or resistant attachment) were compared to children with secure attachment, excluding children with disorganized attachment. Model 1 was adjusted for: total ICV (total intracranial volume), child age at brain MRI scan, child sex. Model 2 was additionally adjusted for: maternal education, maternal psychiatric symptoms and alcohol use during pregnancy. Global structural brain measures were not adjusted for total ICV. All brain outcomes are standardized.

8

No Robust Evidence for Brain Volumetric Correlates of Resilience in Two Independent Cohort Studies

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ABSTRACT

Background: Childhood adversities have been associated with long-lasting brain morphological differences and poor psychological outcomes over the lifespan. Evidence of protective factors counteracting the detrimental effects of childhood adversity on neurobiology is scarce.

Methods: We examined the interplay of childhood adversity with a range of protective factors in relation to brain morphology in two independent longitudinal birth cohorts, the Generation R Study (N=3,008) and the Mannheim Study of Children at Risk (MARS) (N=179). Cumulative exposure to 12 adverse events (such as physical and sexual abuse), and the presence of protective factors, including child temperament, cognition, self-esteem, friendship quality, and maternal sensitivity were assessed at different time points during childhood in both cohorts. Anatomical scans were acquired at the ages of 9-11 years in Generation R and at 25 years in MARS, capturing different developmental stages and allowing us to address the interaction between adversity and protective factors on short- and long-term brain differences.

Results: Childhood adversity was related to smaller cortical grey matter, cerebral white matter and cerebellar volumes in Generation R, with similar effect sizes observed for cerebellar volume in MARS. Some interaction effects between adversity and protective factors were found on the medial orbitofrontal cortex and amygdala in only either one of the two cohorts, but no interaction effect survived correction for multiple comparisons.

Conclusions: We found no consistent evidence for interaction effects between protective factors and childhood adversities on broad brain structural measures. The small interaction effects found in either children or adults warrant further investigation.

INTRODUCTION

The cumulative exposure to adversities, such as parental loss and physical abuse, has been robustly related to long-lasting psychiatric problems throughout life, including behavior, mood, anxiety, and substance disorders (Kessler et al., 2010; McLaughlin et al., 2019), accounting for about 30% of these psychopathologies in adulthood (Kessler et al., 2010). Evidence also suggests biological consequences of early-life adversities, with multiple studies showing brain morphological differences in individuals exposed to childhood adversity (McLaughlin et al., 2019; Monninger et al., 2019). Adversity has been associated with smaller global brain volumes and with volumetric differences in brain regions involved in stress response and the regulation of emotions; including the amygdala, hippocampus, anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC) (see reviews: Holz et al. (2020), Bick and Nelson (2016), and McLaughlin et al. (2019)). Some of these findings are described in children and adults, possibly supporting a long-term effect of childhood adversities (Holz et al., 2020).

While there is evidence for the relation between early-life adverse events and brain structure, little is known about protective factors that could counteract these effects. These factors, also termed "resilience factors" (Ellis et al., 2017), promote psychological resilience, allowing the individual to achieve healthy psychological outcomes despite exposure to adversity (McEwen et al., 2015). Protective factors, including optimism, positive coping styles, maternal sensitivity, high caregiver support, and having close social contacts, were associated with neural morphological differences, particularly in areas that are also related to adversity and that are implicated in emotion, cognition, stress regulation and affective processing (Dolcos et al., 2016; Holz et al., 2016; Holz et al., 2020; Kok et al., 2015; Luby et al., 2019; Taebi et al., 2020). However, whereas some studies provided initial evidence for an adversity-counteracting effect of maternal sensitivity (Holz et al., 2021; Morgan et al., 2014) and self-esteem (Wang et al., 2016), others showed no buffering effect of high caregiver support and environmental enrichment (Luby et al., 2019; Mackes et al., 2020) on the brain outcomes. Further studies and analyses of additional protective factors are needed, considering the limited and conflicting evidence regarding the neural correlates of resilience.

Brain development goes through rapid and substantial changes during childhood, including synaptogenesis, dendritic growth, and myelination (Lyall et al., 2015). In this dynamic maturation process, neuroplasticity is increased, and environmental influences may have lasting effects (White, 2019). Thus, we analyzed whether adversities interact with various protective factors during childhood to shape brain morphology. Based on previous literature, we examined the cortical grey matter, cerebral white matter, cerebellum, amygdala, hippocampus, ACC, and medial OFC volumes. We hypothesized smaller volumes of these structures would be observed in participants with childhood adversi-

ties, and that this association would be buffered in the presence of protective factors. We used data from two longitudinal independent cohorts, Generation R (N= 3,008 in the analyses) and the Mannheim Study of Children at Risk (MARS, N= 179 in the analyses) to address our research question with a generalizability approach, with the aim of exploring whether similar findings would be observed across different developmental stages. Whereas Generation R is a population-based cohort, MARS is oversampled for high-risk participants, and brain outcomes were assessed during childhood in Generation R and in adulthood in MARS. Both birth cohorts provided a rich set of data on adversities and protective factors assessed during childhood, which allowed a substantial harmonization across cohorts. Given the differences in ages, sample size and sampling frame, our analyses were not performed with a replication approach.

METHODS

Participants

We used data from two ongoing prospective birth cohort studies, the Generation R Study and MARS (Figure 1).

The Generation R Study is a population-based cohort study that follows the development of children in Rotterdam, the Netherlands (Kooijman et al., 2016). Pregnant women with an expected delivery date between April 2002 and January 2006 were invited to participate, and 9,778 women were enrolled in the study (response rate at birth: 61%). The study was approved by the Medical Ethical Committee of the Erasmus Medical Center, and all parents gave written informed consent. Overall, 6,882 children had information available on at least 50% of the childhood adversity measures. Among this sample, structural brain magnetic resonance imaging (MRI) scans were obtained in 3,925 nine-to-eleven-year-old children (White et al., 2018). We excluded children with poor image quality data (N= 763), and one sibling selected at random from each sibling pair to avoid bias due to paired data (N= 154). In total, 3,008 children were included in the analyses (Figure S1).

MARS is a birth cohort that follows the development of participants since early life to study the long-term outcomes of early risk factors (Laucht et al., 2000). Inclusion of infants was based on a two-factorial design (factor one as the presence of obstetric complications, and factor two as psychosocial adversity) to enrich the sample with infants exposed to early psychosocial and biological risk factors. Only firstborn children with predominantly European descent (>99%) and German-speaking parents were included. MARS was approved by the Ethics Committee of the University of Heidelberg, and all participants gave written informed consent. In total, 384 infants born between 1986 and 1988 were recruited from two obstetric and six children's hospitals in the Rhine-

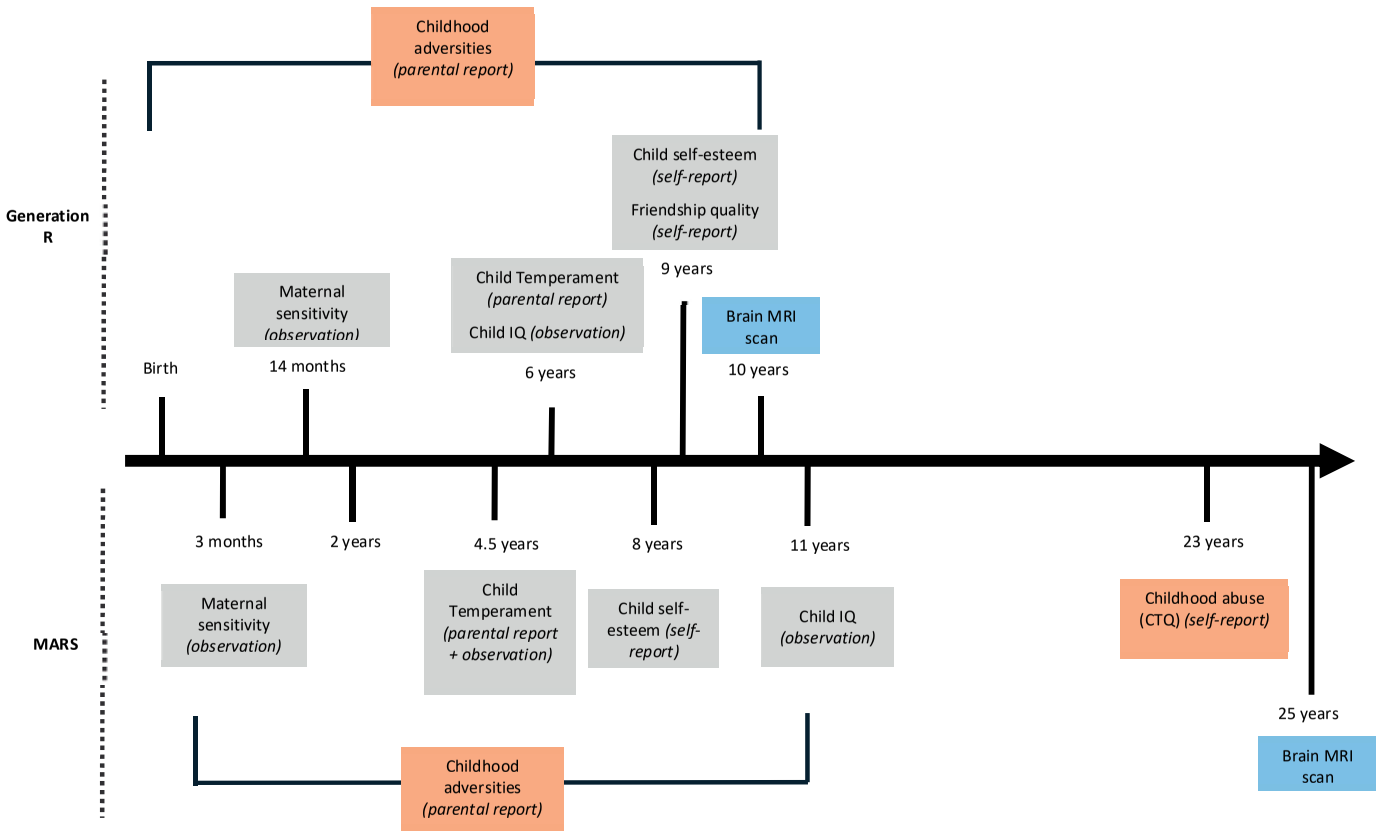

Figure 1. Timeline of data collection for the main variables of interest in Generation R and MARS

Note. In grey: protective factors. In blue: MRI scans. In orange: adversity measures. All measures were prospectively collected except for: childhood abuse (CTQ) in MARS, and some childhood adversity questions in Generation R (for more detail see Table 2).

Neckar Region of Germany. Among these participants, 18 (4.7%) were excluded because of severe disabilities, and 57 (14.8%) were dropouts. From the 309 participants included in the 25-year assessment, structural brain MRI data were collected in a subsample of right-handed participants with no current psychopathology. In total, 179 participants were included in our study sample (Figure S2; Supplement).

Measures

Childhood adversity

In Generation R and MARS, a sum score of childhood adversities was constructed based on the occurrence of 12 adverse events during childhood that were similar across both cohorts: early parenthood, one-parent family at child birth, unwanted pregnancy, parental psychopathology, poverty, parent's death, family relationship problems, parental divorce/separation, unemployment, physical abuse, psychological abuse, and sexual abuse. Data on these events were collected primarily during childhood in both cohorts (Table S1; Supplement).

Childhood protective factors

Protective factors were selected based on previous research (Ellis et al., 2017; Holz et al., 2020; Wang et al., 2016), and were measured during childhood in both cohorts. We included child temperament, child intelligence quotient (IQ), child self-esteem, and maternal sensitivity. Friendship quality was only included in Generation R since no comparable measure was available in MARS (Table S2; Supplement).

Child temperament was reported by the main caregiver in Generation R at child age 6 years, based on the Very Short Form of the Children's Behavior Questionnaire (Putnam & Rothbart, 2006) (dimensions: negative affectivity (reversed in our analyses to facilitate interpretation), surgency/extraversion, and effortful control). In MARS, child temperament was based on a standardized parent interview and observations of the child in familiar and unfamiliar settings at age 4.5 years, using rating scales and an interview adapted from Thomas et al. (1968) (Factors extracted: easy-difficult trait and self-control).

Child IQ was assessed with a non-verbal cognition test in both cohorts, measured at 6 years in Generation R children using the Snijders-Oomen Nonverbal Intelligence Test (SON-R 2.5-7) (Tellegen et al., 1998), and at 11 years in MARS, with the Culture Fair Intelligence Test (CFT-20) (Cattell, 1960).

Child self-esteem was reported by children at age 9 years in Generation R and at age 8 years in MARS. In Generation R, global self-esteem was assessed based on the Dutch version of Harter's Self-Perception Profile for Children (Veerman et al., 1997), with an adapted question format based on Wichstraum (1995). In MARS, global child

self-concept (referred to as self-esteem) was assessed using the German version of the Perceived Competence Scales (Asendorpf & Van Aken, 1993; Harter & Pike, 1984).

Maternal sensitivity was observed in both cohorts. In Generation R, maternal sensitivity was examined in a subsample of children with Dutch national origin (N= 383 in these analyses) during the 14-month laboratory visit, and rated using Ainsworth's scales (Ainsworth et al., 1974). In MARS, the interaction between the mother and the 3-month-old infant was coded using the Mannheim Rating System for Mother-Infant Interaction (Esser et al., 1989). We included adequate maternal stimulation as a measure of maternal sensitivity (Holz et al., 2018; Holz et al., 2021) and infant responsiveness was added as a covariate in these analyses to assess maternal behavior independent of the degree of child responsiveness (Holz et al., 2018).

Friendship quality was assessed at child age 9 years in Generation R. Children rated the quality of their best friendship based on an adapted version of the Friendship Quality Questionnaire (FQQ) (Parker & Asher, 1993).

Brain Morphology

Generation R.

At 9-to-11 years of age, children underwent a neuroimaging scanning session, with a 3-Tesla MRI scanner (MR750w, General Electric, Milwaukee, WI, USA) using an 8-channel receive-only head coil (White et al., 2018). T_1-weighted structural images were obtained with a coronal inversion recovery fast spoiled gradient recalled sequence (IR-FSPGR BRAVO) (ARC acceleration factor= 2, Repetition time= 8.77 ms, Echo time= 3.4 ms, Inversion time= 600 ms, Flip angle= 10°, Field of view= 220x220, Acquisition matrix= 220x220, Slice thickness= 1 mm, Number of slices= 230).

MARS.

At 25 years of age, participants underwent the neuroimaging data collection, with a 3-Tesla MRI scanner (Magnetom TRIO, Siemens, Erlangen, Germany) using a 12-channel head coil. The 1x1x1 mm^3 T_1-weighted MRI scans were acquired with the following parameters: Number of slices= 192, Matrix= 256x256, Repetition time= 2300 ms, Echo time= 3.03 ms, 50% distance factor, Field of view= 256x256x192 mm^3, Flip angle= 9° (Holz et al., 2015; Monninger et al., 2019).

Anatomical data analysis - Generation R and MARS.

Neuroimaging data were processed using the FreeSurfer analysis suite (v.6.0) (Fischl, 2012). Briefly, cortical reconstruction (removal of non-brain tissue, correction of voxel intensities, voxels segmentation into white and grey matter and cerebral spinal fluid, and generation of surface-based models of white and grey matter) and volumetric seg-

mentation were performed. Global and regional brain volume metrics were extracted and cortical vertices were automatically labelled based on the Desikan-Killiany atlas (Desikan et al., 2006).

Regions of interest (ROIs).

Based on previous literature (Holz et al., 2020), we examined the cortical grey matter, cerebral white matter, cerebellum, amygdala, hippocampus, left and right ACC, and left and right medial OFC volumes (ACC and medial OFC based on the Desikan-Killiany atlas (Desikan et al., 2006)). The ACC measure was constructed as the sum of the rostral and caudal ACC. Left and right ACC and medial OFC were examined separately given recent evidence of cortical structural asymmetry (Kong et al., 2018). As in previous studies (Gehred et al., 2021; Luby et al., 2019), we averaged amygdala and hippocampal volumes across hemispheres, to reduce the number of tests in our main analyses and since we had no a priori hypothesis for laterality-specific effects. Left and right amygdala and hippocampus volumes, and the ACC and medial OFC surface area, were studied in sensitivity analyses. Cortical surface area was examined considering that it is relatively less developed than cortical thickness at birth, and that while cortical thickness is largely established by age 2 years, surface area undergoes substantial developmental changes during childhood accounting for most of the cortical volume increases in this period (Lyall et al., 2015).

Covariates

Covariates were selected based on previous literature (Luby et al., 2019; Monninger et al., 2019; Pulli et al., 2019). Covariates included sex, total intracranial volume (included in analyses of the amygdala, hippocampus, ACC and medial OFC), prenatal smoking (Pulli et al., 2019), maternal national origin (only in Generation R), age at MRI scan (only in Generation R, given only very small age-related effects in early adulthood (Ziegler et al., 2012)), and a measure of obstetric risk including low birth weight (Laucht et al., 2000). Additionally, child responsiveness was adjusted for in analyses with maternal sensitivity in MARS (see Supplement).

Statistical analyses

All analyses were performed with R statistical software (v.4.1.0) (R Core Team, 2020). Pearson correlations were calculated to describe the overall associations across the main variables of interest. Multiple linear regression analyses adjusted for covariates were performed to examine the main effects of childhood adversity and the additive interactions between adversity and protective factors on the brain outcomes. The interaction effects were assessed by including a multiplicative term between cumulative adversity and the protective factor in separate models for each protective factor, implying that sample sizes could vary across analyses (e.g. analyses including maternal sensitivity

performed in N= 383 in Generation R, and N= 173 in MARS. All sample sizes are noted in tables' footnotes).

In sensitivity analyses, we explored an additional measure of our cortical ROIs and the potential moderation by hemisphere laterality and national origin (see Supplement). Specifically, we analyzed the interaction of adversities with the protective factors for: 1) the surface area of the left and right ACC and medial OFC; 2) the left and right amygdala and hippocampus; and 3) the cortical grey matter, cerebral white matter, cerebellar, amygdala, hippocampal, ACC and medial OFC volumes only in Generation R children with mothers of European descent.

We corrected for multiple testing using the false discovery rate (FDR) (Benjamini & Hochberg, 1995) in the main analyses of the association between adversity and the brain outcomes (nine tests per cohort) and in the interaction analyses between adversity and protective factors on the brain outcomes (In Generation R, seven protective factors and nine outcomes: 63 tests; in MARS, five protective factors and nine outcomes: 45 tests). The sensitivity analyses were not corrected for multiple testing as these were exploratory.

All effect estimates were standardized. Analyses in MARS were performed in participants with complete data (due to few missing values). In Generation R, missing values for covariates, childhood adversity, and protective factors (maximum missingness: paternal psychopathology at child age 3 years: 42%, and maternal psychopathology at child age 6 months: 37%) were imputed using the Multivariate Imputation by Chained Equations package (v.3.13.0) (van Buuren & Groothuis-Oudshoorn, 2011), pooling results across 40 imputed datasets. The missingness in the two psychopathology measures in Generation R is largely explained by study design. During child ages 0-4 years, data collection only included participants in northern Rotterdam due to logistical constraints. From child age 6 years onwards, all children from the initial catchment area of Rotterdam were invited to participate in follow-up assessments (Kooijman et al., 2016). Maternal sensitivity was not imputed, as it was assessed in a subsample of Generation R and values were missing for 87.3% of the children. See Supplement for the non-response analyses.

RESULTS

The samples' characteristics are described in Table 1. In total, 70% of participants in Generation R, and 91% in MARS, were exposed to at least one adversity. In both cohorts, the most common adversities were parental psychopathology (Generation R: 32.6%, MARS: 49.2%) and unemployment of both parents (Generation R: 31.6%, MARS: 70.4%) (unemployment in MARS includes job loss and unemployment for more than 3 months) (Table 2).

Table 1. Baseline characteristics of the samples from the Generation R Study and MARS

	Generation R		MARS		
Sample characteristics					
Sex, N (%) female		1516 (50.4)			105 (58.7)
Age at the MRI scan (years), mean (SD)		10.1 (0.58)			25.0 (0.60)
Childhood adverse events, N (%)					
0		892 (29.7)			17 (9.5)
1		810 (26.9)			36 (20.1)
2		515 (17.1)			42 (23.5)
3		359 (11.9)			32 (17.9)
4 or more		432 (14.4)			52 (29.0)
Maternal national origin, N (%)					
European descent		1980 (65.8)			-
Others		1028 (34.2)			-
Maternal smoking, smoking during pregnancy, N (%)		683 (22.7)			40 (22.3)
Protective factors	**Reporter**	**mean (SD)**		**Reporter**	**mean (SD)**
Temperament, at 6 years	MC		**Temperament, at 4.5 years**	Ob +Int	
Temperament - Negative affectivity, reversed		-3.70 (0.83)	Temperament - easy/difficult trait		0.05 (0.96)
Temperament - Surgency		4.42 (0.79)	Temperament - self-control		0.14 (0.92)
Temperament - Effortful control		5.29 (0.68)			
Child non-verbal IQ, at 6 years	Ob	102.95 (14.88)	**Child non-verbal IQ, at 11 years**	Ob	105.68 (11.22)
Child self-esteem, at 9 years	Ch	45.73 (4.26)	**Child self-esteem, at 8 years**	Ch	57.64 (6.36)
Maternal sensitivity, at 14 months*	Ob	0.02 (0.81)	**Maternal stimulation (sensitivity), at 3 months****	Ob	-0.06 (2.60)
Additional cohort-specific measures					
Friendship quality, at 9 years	Ch	24.12 (3.25)	-	-	-

Note. Characteristics of the study sample and protective factors (pooled imputed data in Generation R). Abbreviations: MC: Main caregiver, Ob: Observation, Ch: Child, Int: Interview. In Generation R, N = 3,008. *Maternal sensitivity in Generation R available for N = 383. In MARS, N = 179. **Maternal sensitivity in MARS available for N = 173

Table 2. Prevalence of childhood adversities in the Generation R Study and the MARS

	Generation R Study					MARS				
	Event	Age at reporting	Reporter	Exposed		Event	Age at reporting	Reporter	Exposed	
				N	%*				N	%*
1	Early parenthood	pregnancy	Mother	79	2.6	Early parenthood	3 months	parents	47	26.3
2	One-parent family at child birth	pregnancy	Mother	350	11.6	One-parent family at child birth	3 months	parents	17	9.5
3	Unwanted pregnancy	pregnancy	Mother	39	1.3	Unwanted pregnancy	3 months	parents	24	13.4
4	Parental (maternal or paternal) psychopathology	2 months, 6 months, 3 and 9 years	Mother and partner	982	32.6	Parental (maternal or paternal) psychopathology	3 months, 2, 4.5, 8 and 11 years	parents	88	49.2
5	Poverty	pregnancy	Mother	547	18.2	Poverty	3 months	mother	35	19.6
6	Death of parent	9 years	Caregiver*	28	0.92	Death of parent	2, 4.5, 8 and 11 years	parents	3	1.7
7	Family relationship problems	3, 5, and 9 years	Caregiver*/mother/ partner	663	22.0	Family relationship problems	3 months, 2, 4.5, 8 and 11 years	parents	71	39.7
8	Divorce/separation by age 9 years	3, 5 and 9 years	Mother	663	22.0	Divorce/separation by age 11 years	2, 4.5, 8 and 11 years	parents	44	24.6
9	Unemployment	3 and 9 years	Caregiver*	952	31.6	Unemployment	2, 4.5, 8 and 11 years	parents	126	70.4
10	Physical abuse to child	9 years	Caregiver*	209	6.9	Physical abuse to child	23 years	participant**	5	2.8
11	Psychological abuse to child	9 years	Caregiver*	349	11.6	Psychological abuse to child	23 years	participant**	13	7.3
12	Sexual abuse	9 years	Caregiver*	134	4.5	Sexual abuse	23 years	participant**	3	1.7
	Any category reported			2116	70.3	***Any category reported***			162	90.5

Note. Characteristics described in imputed dataset of Generation R Study. Generation R N = 3,008
MARS N = 179
* Caregiver = Main caregiver. ** Participant = self-report.

Childhood adversity and brain volumes

In Generation R, exposure to adversity was associated with smaller cortical grey matter (β= -0.09, 95% confidence interval (CI) -0.12 to -0.06, p_{uncorr}< 0.001, p_{corr}< 0.001), cerebral white matter (β= -0.07, CI -0.11 to -0.04, p_{uncorr}< 0.001, p_{corr}< 0.001), and cerebellar (β= -0.08, CI -0.12 to -0.05, p_{uncorr}< 0.001, p_{corr}< 0.001) volumes. In MARS, the latter showed a similar effect size (β= -0.11, CI -0.24 to 0.01), but did not reach statistical significance (p_{uncorr}= 0.08). In Generation R, adversity was also related to larger left medial OFC volume (β= 0.04, CI 0.01 to 0.08, p_{uncorr}= 0.01, p_{corr}= 0.02). No other associations were observed for the global and regional, subcortical (amygdala and hippocampus), or cortical (left and right ACC and medial OFC) volumes in either cohort (Table 3).

Childhood adversity, protective factors, and brain volumes

No results from these analyses survived adjustment for multiple testing nor were consistent across cohorts (Table 4). In Generation R, more maternal sensitivity buffered the association of greater adversity levels with smaller right medial OFC volume (β= 0.08, CI 0 to 0.16, p_{uncorr}= 0.04, p_{corr}= 0.90), but this interaction effect was not observed in MARS (β= 0.05, CI -0.04 to 0.14, p_{uncorr}= 0.28) (Figure S3).

Table 3. Associations between cumulative childhood adversity and brain outcomes

	Generation R Study		MARS	
	ß (95%CI)	p-value	ß (95%CI)	p-value
Brain outcomes				
Global and regional brain outcomes				
Cortical grey matter volume	-0.09 (-0.12; -0.06)	< 0.001*	0 (-0.12; 0.12)	0.96
Cerebral white matter volume	-0.07 (-0.11; -0.04)	< 0.001*	-0.01 (-0.14; 0.12)	0.88
Cerebellar volume	-0.08 (-0.12; -0.05)	< 0.001*	-0.11 (-0.24; 0.01)	0.08
Subcortical outcomes				
Amygdala	-0.01 (-0.04; 0.02)	0.59	0.06 (-0.04; 0.16)	0.23
Hippocampus	0 (-0.03; 0.04)	0.79	0.06 (-0.06; 0.17)	0.33
Cortical regions				
Left ACC volume	0.02 (-0.01; 0.06)	0.18	-0.01 (-0.13; 0.12)	0.93
Right ACC volume	0 (-0.04; 0.03)	0.89	0.02 (-0.11; 0.16)	0.74
Left medial OFC volume	0.04 (0.01; 0.08)	0.01*	0 (-0.12; 0.11)	0.95
Right medial OFC volume	0 (-0.03; 0.03)	0.99	0.01 (-0.11; 0.12)	0.93

Note. Model adjusted for sex, total intracranial volume (only in subcortical and cortical regions), prenatal smoking, maternal national origin (only in Generation R), age at the MRI scan (only in Generation R), and obstetric risk.

Adversity and brain outcomes were standardized. Amygdala and hippocampus volumes are the mean volumes across left and right hemisphere. Abbreviations: ACC: Anterior cingulate cortex, OFC: Orbitofrontal cortex

Generation R N = 3,008

MARS N = 179

*p-values that survived adjustment for multiple testing (including all regions of interest, method: FDR).

Table 4. Interaction between protective factors and cumulative adverse events in relation to brain outcomes

	Global and regional brain outcomes						*Subcortical outcomes*			
	Cortical grey matter volume		Cerebral white matter volume		Cerebellar volume		Amygdala		Hippocampus	
	ß (95%CI)	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**
Generation R Study										
Temperament - Negative affectivity, reversed	-0.01 (-0.05; 0.02)	0.49	0 (-0.03; 0.04)	0.89	0.01 (-0.03; 0.05)	0.58	0 (-0.03; 0.03)	0.85	-0.01 (-0.04; 0.02)	0.56
Temperament - Surgency	0.02 (-0.02; 0.06)	0.30	0.01 (-0.03; 0.04)	0.77	0.02 (-0.02; 0.05)	0.35	0.01 (-0.02; 0.04)	0.53	-0.01 (-0.04; 0.02)	0.54
Temperament - Effortful control	-0.02 (-0.05; 0.01)	0.24	-0.01 (-0.04; 0.03)	0.65	0.01 (-0.03; 0.04)	0.73	0 (-0.03; 0.04)	0.79	0.02 (-0.01; 0.05)	0.27
Child non-verbal IQ	-0.02 (-0.05; 0.01)	0.20	-0.02 (-0.05; 0.02)	0.28	-0.01 (-0.04; 0.03)	0.60	-0.02 (-0.05; 0.01)	0.29	-0.02 (-0.05; 0.01)	0.28
Child self-esteem	0.01 (-0.03; 0.04)	0.70	0.01 (-0.02; 0.04)	0.48	0 (-0.03; 0.04)	0.78	0 (-0.03; 0.03)	0.95	0 (-0.03; 0.04)	0.75
Maternal sensitivity*	0.05 (-0.04; 0.13)	0.29	0 (-0.09; 0.08)	0.97	-0.01 (-0.10; 0.07)	0.73	0.05 (-0.02; 0.12)	0.18	0 (-0.07; 0.08)	0.93
Friendship quality	0.02 (-0.01; 0.06)	0.24	0.03 (0; 0.07)	0.09	0.04 (0; 0.07)	0.052	-0.02 (-0.05; 0.01)	0.19	-0.01 (-0.05; 0.02)	0.38
MARS										
Temperament										
Temperament - easy/difficult trait	-0.01 (-0.14; 0.11)	0.81	0.01 (-0.12; 0.14)	0.84	0.01 (-0.12; 0.14)	0.86	-0.04 (-0.14; 0.06)	0.44	-0.04 (-0.15; 0.07)	0.49
Temperament - self-control	0.03 (-0.07; 0.13)	0.56	0.02 (-0.08; 0.13)	0.66	0.04 (-0.07; 0.14)	0.49	0 (-0.08; 0.08)	0.98	-0.01 (-0.10; 0.08)	0.84
Child non-verbal IQ	0.01 (-0.10; 0.12)	0.89	0.04 (-0.08; 0.16)	0.47	-0.03 (-0.15; 0.09)	0.61	-0.08 (-0.18; 0.01)	0.07	-0.01 (-0.11; 0.10)	0.91
Child self-esteem	-0.03 (-0.16; 0.10)	0.65	0.02 (-0.12; 0.16)	0.78	0.08 (-0.06; 0.22)	0.26	0.10 (-0.01; 0.21)	0.06	0.09 (-0.03; 0.21)	0.16
Maternal stimulation (sensitivity)**	0.02 (-0.07; 0.12)	0.65	0.05 (-0.05; 0.15)	0.33	-0.01 (-0.11; 0.09)	0.90	0.01 (-0.07; 0.09)	0.77	0.03 (-0.06; 0.12)	0.48

Table 4. Interaction between protective factors and cumulative adverse events in relation to brain outcomes (continued)

	Left ACC		Right ACC		Left medial OFC		Right medial OFC	
	β (95%CI)	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**
Generation R Study								
Temperament - Negative affectivity, reversed	0 (-0.03; 0.04)	0.88	-0.02 (-0.06; 0.02)	0.27	-0.02 (-0.06; 0.01)	0.17	-0.02 (-0.05; 0.01)	0.28
Temperament - Surgency	0 (-0.03; 0.03)	0.95	0.01 (-0.03; 0.04)	0.73	0 (-0.04; 0.03)	0.90	0 (-0.03; 0.04)	0.97
Temperament - Effortful control	-0.01 (-0.05; 0.02)	0.39	-0.01 (-0.05; 0.03)	0.61	-0.01 (-0.04; 0.02)	0.46	-0.01 (-0.04; 0.02)	0.60
Child non-verbal IQ	-0.02 (-0.06; 0.01)	0.16	-0.01 (-0.05; 0.02)	0.49	-0.01 (-0.04; 0.03)	0.70	-0.01 (-0.04; 0.02)	0.58
Child self-esteem	-0.01 (-0.04; 0.02)	0.51	0 (-0.03; 0.03)	0.96	0 (-0.03; 0.03)	0.97	0.02 (-0.01; 0.05)	0.25
Maternal sensitivity*	0 (-0.08; 0.08)	0.94	0.03 (-0.06; 0.12)	0.54	0.05 (-0.03; 0.12)	0.21	0.08 (0; 0.16)	0.04
Friendship quality	-0.03 (-0.06; 0)	0.08	0.01 (-0.03; 0.04)	0.61	0 (-0.03; 0.03)	0.92	0.01 (-0.02; 0.04)	0.41
MARS								
Temperament								
Temperament - easy/difficult trait	0.06 (-0.07; 0.18)	0.35	-0.02 (-0.15; 0.12)	0.79	-0.14 (-0.25; -0.03)	0.02	-0.10 (-0.21; 0.01)	0.07
Temperament - self-control	0.01 (-0.10; 0.11)	0.92	0.04 (-0.07; 0.15)	0.47	-0.03 (-0.13; 0.06)	0.46	-0.03 (-0.12; 0.06)	0.49
Child non-verbal IQ	-0.05 (-0.16; 0.07)	0.43	0.05 (-0.07; 0.18)	0.41	0.01 (-0.09; 0.11)	0.85	-0.02 (-0.13; 0.08)	0.67
Child self-esteem	0.02 (-0.11; 0.16)	0.75	0.07 (-0.08; 0.22)	0.35	0.02 (-0.10; 0.14)	0.76	-0.06 (-0.18; 0.07)	0.37
Maternal stimulation (sensitivity)**	0.01 (-0.09; 0.11)	0.87	-0.02 (-0.12; 0.09)	0.77	-0.02 (-0.11; 0.07)	0.73	0.05 (-0.04; 0.14)	0.28

Note. Predictors included: cumulative adversity, protective factor (specific for each model), sex, total intracranial volume (only in subcortical and prefrontal regions), prenatal smoking, maternal national origin (only in Generation R), age at the MRI scan (only in Generation R), obstetric risk, and the interaction term between each protective factor and cumulative adversity. Analyses with maternal sensitivity predictors in MARS additionally adjusted for child responsiveness. Analyses with maternal sensitivity in Generation R not adjusted for maternal national origin. Negative affectivity scores in Generation R were reversed.

All brain outcomes and adversity and protective factors were standardized. Amygdala and hippocampus volumes are the mean volumes across left and right hemisphere. Abbreviations: ACC: Anterior cingulate cortex, OFC: Orbitofrontal cortex.

Generation R N = 3,008. *Analyses with maternal sensitivity performed in N = 383.

MARS N = 179. **Analyses with maternal sensitivity performed in N = 173.

Adjustment for multiple testing (including all regions of interest, method: FDR): In Generation R and in MARS, no interaction survived.

In MARS, easy/difficult temperament moderated the association between childhood adversity and left medial OFC volume (β= -0.14, CI -0.25 to -0.03, p_{uncorr}= 0.02, p_{corr}= 0.79), such that childhood adversity was associated with larger left medial OFC volumes in children with a more difficult temperament, while it was associated with smaller left medial OFC volumes in children with an easy temperament (Figure S4). We found no interaction between adversity and child temperament on the medial OFC volumes in Generation R.

Sensitivity analyses

These analyses were exploratory and thus were not adjusted for multiple testing. First, we analyzed the interaction of childhood adversity and protective factors on the surface area of the cortical ROIs. In MARS, maternal stimulation buffered the association between childhood adversity and smaller right medial OFC surface area (β= 0.09, CI 0.01 to 0.17, p= 0.03). This interaction was not found in Generation R (β= 0.04, CI -0.03 to 0.12, p= 0.27) (Figure S5; Table S3).

Second, we examined the interaction of adversity with the protective factors separately for the left and right amygdala and hippocampus (Table S4). There was an interaction between adversity and child self-esteem on the right amygdala volume in MARS (β= 0.13, CI 0.02 to 0.24, p= 0.02), such that childhood adversity was associated with smaller right amygdala in participants with low self-esteem, but with larger amygdala in participants with high self-esteem. In Generation R, this interaction was not found (β= 0, CI -0.04 to 0.03, p= 0.89) (Figure S6).

Finally, we explored the interaction of adversity with protective factors on all main outcomes among the subsample of children with mothers of European descent in Generation R (Tables S5 and S6). These analyses were performed for all protective factors, except maternal sensitivity, as this variable was originally assessed only in mothers of Dutch national origin. Consistent with the main analyses, no interaction effects were observed in this subsample.

DISCUSSION

Using two prospective birth cohorts, we investigated the moderating effects of various protective factors on the association between childhood adversity and brain morphology. Childhood adversity was associated with smaller global brain volumes in childhood, but not in adulthood. Also, a negative association of adversity with cerebellar volumes was apparent in both cohorts, although only significant in Generation R. However, there was little evidence for broad brain volumetric differences associated with the interaction of adversity and the protective factors. Across analyses for multiple protective fac-

tors and various ROIs, no interaction effect survived multiple testing correction. Small interaction effects pertaining to the medial OFC and amygdala were found in either childhood or adulthood and may warrant further investigation.

Childhood adversity was associated with smaller cortical grey matter and cerebral white matter volumes in children but not in adults, and with smaller cerebellar volumes apparent in both age groups. Interestingly, similar results have been described before; e.g., smaller cortical gray matter, white matter, and cerebellar volumes in children and adolescents exposed to early-life adversity (Bick & Nelson, 2016); and a relation between adversity and smaller cerebellar grey matter volumes in adults (Gehred et al., 2021). However, this literature is not entirely consistent (e.g. Gehred et al. (2021)) and it is largely based on *severe* adversities like institutional rearing (Bick & Nelson, 2016). The similar direction for the cerebellar findings in both age periods could reflect the long-term adversity effects, since the cerebellum has an extended postnatal development and is related to neurodevelopmental psychopathologies like autism and schizophrenia (Tiemeier et al., 2010). Regarding our analyses on the interaction of childhood adversity with the protective factors, we found a consistent direction of interaction effects in both cohorts between childhood adversity and maternal sensitivity on the right medial OFC, with uncorrected significant analyses for the medial OFC volume in Generation R and for the medial OFC surface area in MARS. While the lack of equivalent findings across cohorts could signal unrelated mechanisms, it is interesting that both analyses showed a potential buffering effect of maternal sensitivity on the association between adversity and smaller right medial OFC (volume in Generation R and surface area in MARS). The uniqueness and robustness of the observational sensitivity measures, the prospective data collection in infants, and the standardized brain morphology assessments further support these findings. In fact, a protective effect of early maternal care has previously been suggested in participants with high familial risk for psychopathology in MARS, resulting in a faster amygdala habituation, altered reward sensitivity, and fewer cases of attention deficit hyperactivity disorder (Holz et al., 2018; Holz et al., 2021). Furthermore, a potential morphometric susceptibility of the OFC to early-life adversity that may confer risk for externalizing (Holz et al., 2015) and internalizing psychopathology (Monninger et al., 2019) has been demonstrated. Additionally, the cortical surface area has a period of rapid development during childhood, largely driving the parallel cortical volume growth (Lyall et al., 2015). Since cortical neurons do not regenerate, any evidence of resilience to adversity in these cortical measures likely reflects a reshaping of existing brain networks (White, 2019). Future studies should replicate and evaluate in depth this interaction using larger neurodevelopmental cohort studies.

Although the overall differences across cohorts may be interpreted as less enduring interaction effects of adversity and protective factors, the cohorts were not used for replication but instead to aid in the generalizability of results. The same mechanisms

that could explain an age-related variation in the association of adversity with brain morphology could also underlie the specific findings observed for resilience. For example, amygdala and hippocampal volumes peak in preadolescence (Uematsu et al., 2012), the time of the brain assessment in Generation R. Therefore, an interaction between adversity and self-esteem on right amygdala volume (as observed in MARS) may not be apparent in childhood, when amygdala volume may be increasing in some children, while decreasing in others. Thus, findings should be considered within the framework of typical brain development specific for each age group. Similarly, although not examined here, the age at the adversity exposure may also influence results, because neuroanatomical vulnerability to environmental factors may be heightened for specific structures in particular sensitive periods (White, 2019).

The lack of robust interaction effects was contrary to our hypothesis, but it is not unexpected. Bonanno (2021) recently described the "resilience paradox," outlining that despite the numerous proposed protective factors, research fails to identify robust evidence for a link between protective factors and the resilience outcomes. Furthermore, this seems to hold true across distinct modelling strategies (Bonanno, 2021). One explanation for the limited evidence in our study and previous research is the lack of stability in the protective factors; that is, people change their behavior in response to different situations and across time (Bonanno, 2021). For example, in children with psychological resilience, protective factors are suggested to specifically manifest in adverse situations, rather than in nonstressful circumstances (*sensitization hypothesis*) (Ellis et al., 2017), highlighting the need of addressing the protective factors' role in models that consider the presence of adversities. Additionally, adversity may shape the protective factors (*specialization hypothesis*) (Ellis et al., 2017), and vice versa, as shown by Rakhshani and Furr (2021). In our study, we assessed protective factors that would be present during the adversity exposure period, to address the moderation effect on the adversity influences. Yet, resilience is by nature a dynamic concept (Holz et al., 2020), and its study will likely benefit from modelling the trajectory of protective factors parallel to that of the adversities.

Additionally, although brain volumetric differences have been observed in relation to childhood adversity and, separately, to protective factors (Holz et al., 2020; McLaughlin et al., 2019), the interaction effects may be smaller, thus requiring larger neurodevelopmental samples to be detected (Maxwell & Delaney, 2004). Also, current research is largely based on standard, broad volume measures. Interaction effects may be focal (e.g., in amygdala sub-regions), and therefore not detectable with mean volumes. Furthermore, the interaction of adversity and protective factors could be more related to the degree of brain adaptability, rather than to volumetric differences per se (Shaw et al., 2006). In fact, a study demonstrated a relation between greater intellectual ability and a more plastic brain cortex (Shaw et al., 2006), and considering that resilience is often defined as the (healthy) *adjustment* to challenges (Bonanno, 2021), future studies should

use repeated brain measures to determine whether resilience is reflected in the degree and characteristics of the brain *adaptability* (McEwen et al., 2015).

Our study contributes with preliminary views into the neuroanatomical correlates of the interplay between adversity and protective factors. Overall, there were no strong interaction effects despite the thorough examination of several protective factors, a rich measure of cumulative adversity, the use of data collected at multiple time points during childhood, and the reasonably large sample size. Importantly, we investigated brain volumes in childhood and adulthood, and we aligned adversity, protective factors, and brain measures across two independent cohorts. Although our approach facilitates qualitative comparisons between a risk-based and a population-based sample, we note that the differences across cohort characteristics impede an interpretation of the results with a replication perspective.

Some additional limitations need to be considered. First, we may have insufficient power to capture small, clinically relevant interaction effects, especially in MARS, in which the high adversity exposure may have narrowed the variability of adversity. We aimed to counteract this limitation by addressing cumulative adversities as a continuum, rather than as a dichotomous score. Further, given the early stage of the literature on protective factors, adversity and brain morphology, prior evidence was insufficient to perform a power calculation and our results should only be regarded as preliminary evidence. Second, information on some adversities and protective factors in Generation R were collected during the same data collection wave as the MRI, thus our results may represent cross-sectional interaction effects. Third, some adversities were retrospectively reported. Although a potential cause of recall bias, we included these measures due to the relevance of the events (e.g., sexual abuse). Furthermore, we assessed mainly objective adverse events, like death or divorce, for which agreement between prospective and retrospective reports is higher compared to that of subjective events (Baldwin et al., 2019). Finally, we acknowledge that any observed interaction effect does not imply causality. Our results may also be explained by reverse causality (i.e., brain morphology determining the protective factors and the specific role of these factors in the interaction with adversity), or by familial neurobiological features that are heritable, determine the protective factors, and are simultaneously non-randomly distributed across adversity occurrence.

Our findings offer initial insights into the neurobiology of resilience and may guide future research investigating the interplay between adversity and protective factors. Overall, our results suggest that resilience to childhood adversity as examined here may not manifest in broad brain volumetric differences in childhood and adulthood. Brain structural characteristics of the interaction between adversity and protective factors are likely to be focal and small. Future studies analyzing larger neurodevelopmental samples and repeated parallel measures of adversity, protective factors, and brain morphology may prove useful as we begin to uncover the neurobiological substrates of resilience.

DATA AVAILABILITY

The data included in the current study are not publicly available due to legal and ethical restrictions. For access to Generation R and MARS, researchers can send their request to Vincent Jaddoe (v.jaddoe@erasmusmc.nl) and to the ethics committee of the University of Heidelberg, respectively.

CONFLICTS OF INTEREST

TB served in an advisory or consultancy role for Actelion, Hexal Pharma, Lilly, Lundbeck, Medice, Novartis and Shire. He received conference support or speaker's fees from Lilly, Medice, Novartis and Shire. He has been involved in clinical trials conducted by Shire and Viforpharma. He received royalties from Hogrefe, Kohlhammer, CIP Medien and Oxford University Press.

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All other authors declare no potential conflicts of interest.

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SUPPLEMENTARY MATERIAL

Supplementary Figures:

Figure S1. Flowchart of sample selection in the Generation R Study.

Figure S2. Flowchart of sample selection in MARS.

Figure S3. Interaction of childhood adversities and maternal sensitivity on the right medial OFC volume.

Figure S4. Interaction of childhood adversities and child temperament (easy/difficult trait) (in Generation R: temperament – Negative affectivity (reversed)) on the left medial OFC volume.

Figure S5. Interaction of childhood adversities and maternal sensitivity on the right medial OFC surface area.

Figure S6. Interaction of childhood adversities and child self-esteem on the right amygdala volume.

Supplementary Tables:

Table S1. Description of childhood adversities in Generation R and MARS.

Table S2. Description of childhood protective factors in Generation R and MARS.

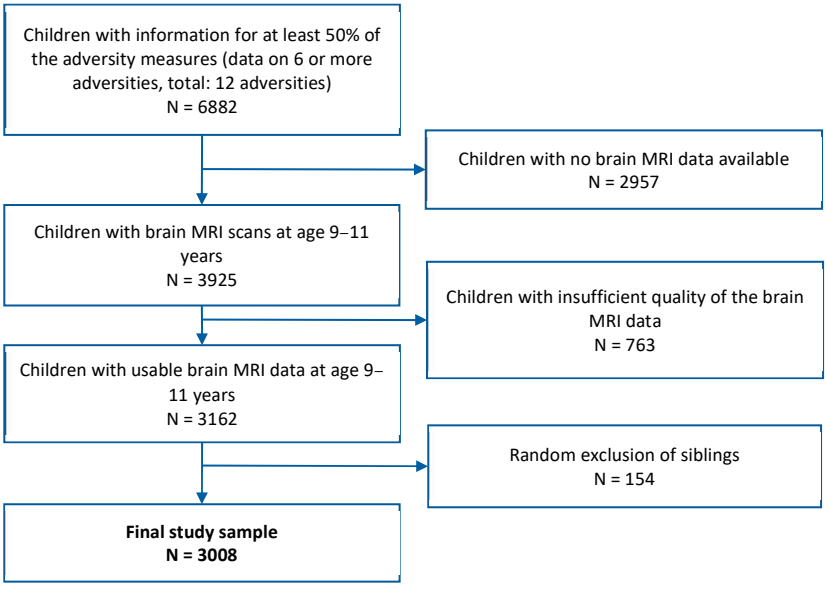
Table S3. Interaction between protective factors and childhood adversity on the surface area of the cortical regions of interest.

Table S4. Interaction between protective factors and childhood adversities in relation to the left and right amygdala and hippocampus.

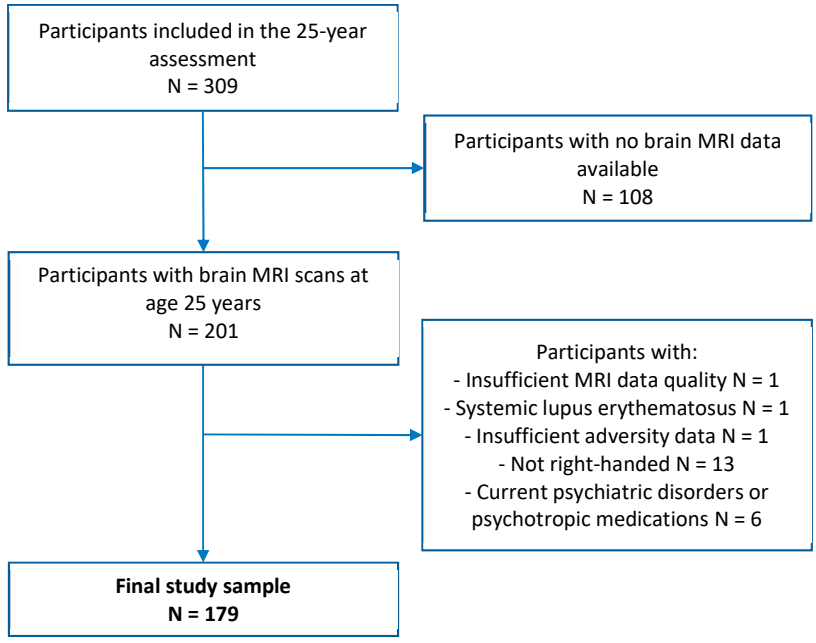
Table S5. Interaction between protective factors and childhood adversities in relation to the brain outcomes in children with mothers of European descent.

Table S6. Interaction between protective factors and childhood adversities in relation to the volumes of the cortical regions of interest in children with mothers of European descent.

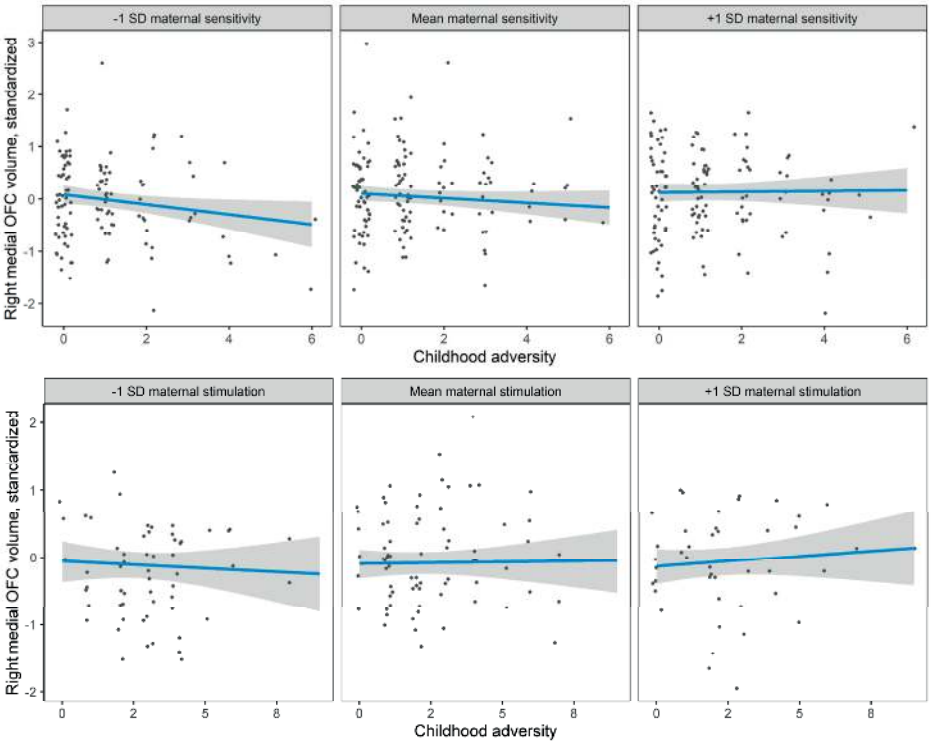
Supplementary Methods



Supplementary Figure 1. Flowchart of sample selection in Generation R.

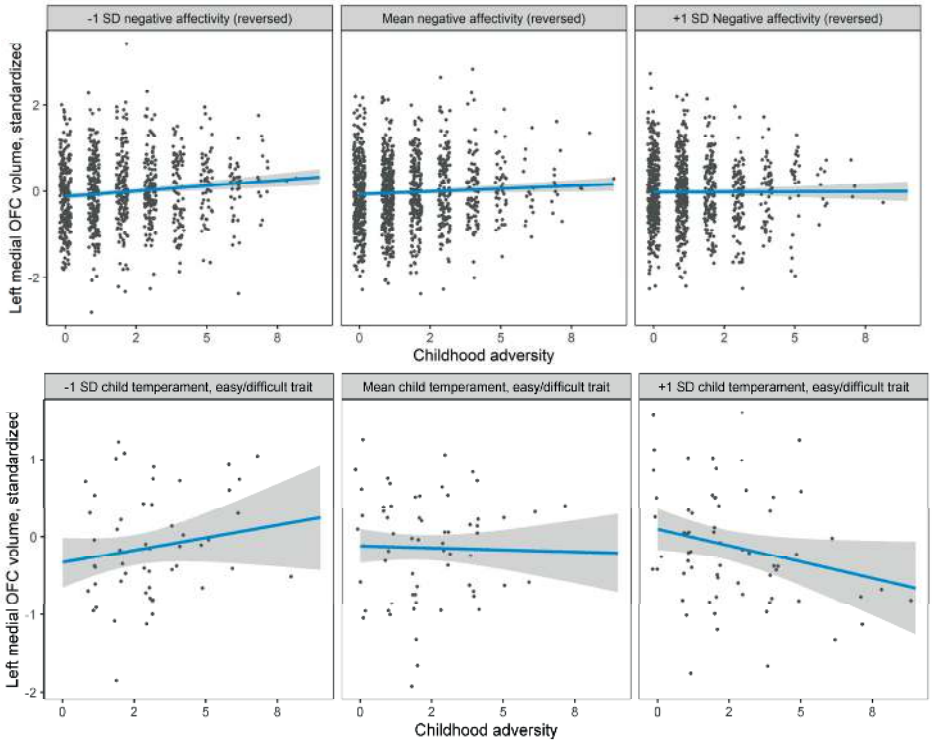


Supplementary Figure 2. Flowchart of sample selection in MARS.



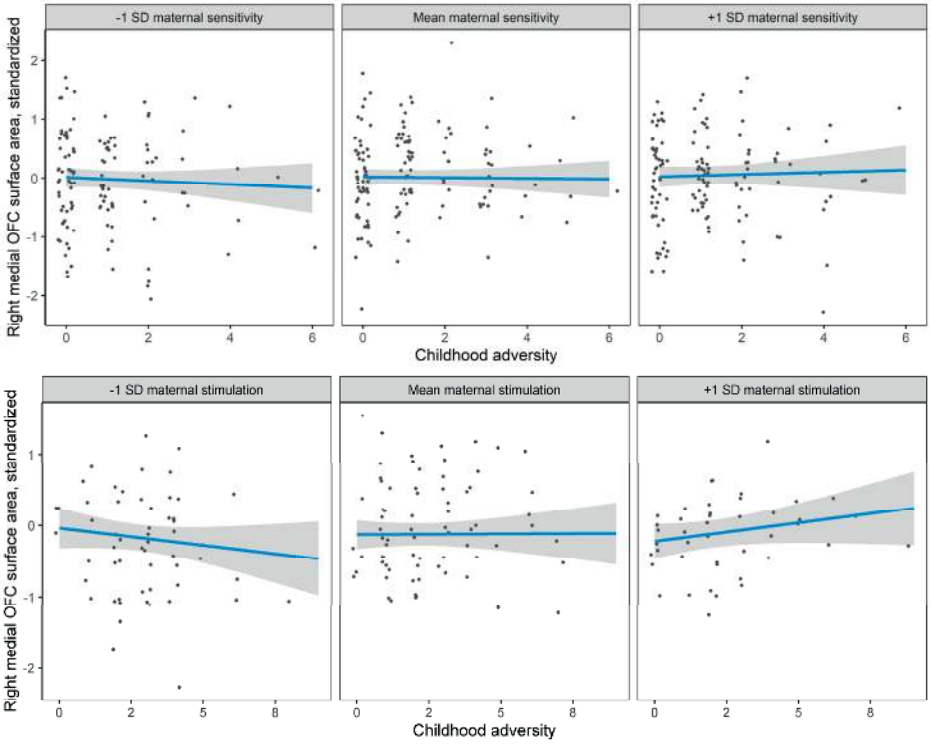
Supplementary Figure 3. Interaction of childhood adversities and **maternal sensitivity** on the **right medial OFC volume.**

Note. The protective factor was standardized. Upper figure: Generation R. Lower figure: MARS.



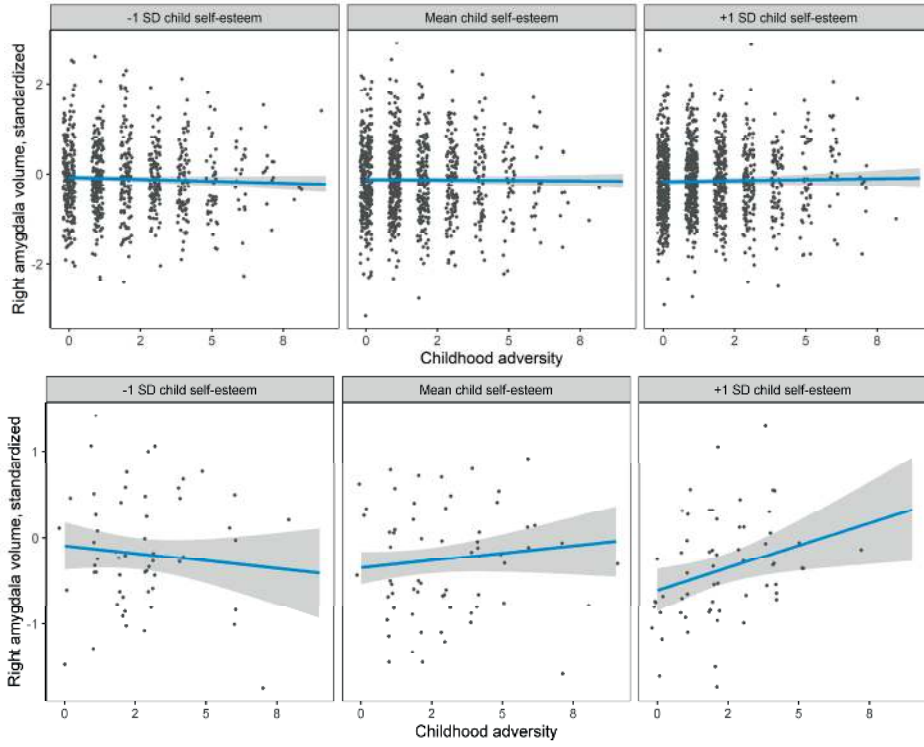
Supplementary Figure 4. Interaction of childhood adversities and **child temperament (easy/difficult trait)** (in Generation R**: temperament - Negative affectivity (reversed)**) on the **left medial OFC volume**.

Note. The protective factor was standardized. Upper figure: Generation R. Lower figure: MARS.



Supplementary Figure 5. Interaction of childhood adversities and **maternal sensitivity** on the **right medial OFC surface area**.

Note. The protective factor was standardized. Upper figure: Generation R. Lower figure: MARS.



Supplementary Figure 6. Interaction of childhood adversities and **child self-esteem** on the **right amygdala volume.**

Note. The protective factor was standardized. Upper figure: Generation R. Lower figure: MARS.

Supplementary Table 1. Description of childhood **adversities** in Generation R and MARS.

	GENR		MARS	
	Event	Description of event	Event	Description of event
1	Early parenthood	Maternal age at child birth < 21 years. Data self-reported via questionnaires during pregnancy.	Early parenthood	Family Adversity Index (FAI): Parental interview assessed in each data collection wave including 11 items, based on an "enriched" family adversity index proposed by Rutter and Quinton (1977). Item used: age of a parent <18 years at child birth or relationship between parents lasting less than 6 months at time of conception.
2	One-parent family at child birth	Pregnant mother reported having no partner.	One-parent family at child birth	FAI item: one-parent family at child birth.
3	Unwanted pregnancy	The pregnancy was unplanned and is unwanted (mixed feelings or not happy about the pregnancy).	Unwanted pregnancy	FAI item: an abortion was seriously considered.
4	Parental (maternal or paternal) psychopathology	Defined as mothers or fathers with symptoms of depression or anxiety. Assessed with the Brief Symptom Inventory (BSI) questionnaire (using the cut-off suggested by the manual). Maternal and/or paternal psychopathology were assessed at various time points during childhood (see Table 2)	Parental (maternal or paternal) psychopathology	Diagnostic interview of psychopathology in each data collection wave. Psychopathology defined as a moderate to severe disorder according to DMS III criteria. Two-stage data collection: first, a complaint inventory was used as a screening instrument. In a second stage, the Structured Clinical Interview for DSM IV was administered covering the preceding 6 months. Interviews were conducted with mothers (and in 40% of cases with fathers as well). If fathers were absent, evaluation of paternal psychopathology relied on maternal information. Assessed at multiple time points during childhood (see Table 2).
5	Poverty	Poverty during pregnancy. Poverty was defined as living below the national low-income threshold, and was based on the number of adults and children living from the same income and the monthly disposable household income (reported via questionnaire).	Poverty	Poverty at the 3-month assessment. Determined by standardized interview with the mother and defined as income level below the risk-of-poverty threshold (threshold was set to 60% of the national median equivalized disposable income adjusted for household size).

Supplementary Table 1. Description of childhood **adversities** in Generation R and MARS. (*continued*)

GENR		MARS	
Event	Description of event	Event	Description of event
6 Death of parent	Parental death by age 9 years. Based on the Life Events Interview. Question: the child's father/mother or the other caregiver is not alive (yes/no).	Death of parent	Parental death by age 11 years. Instrument based on the Munich Events List (MEL) with data collected at multiple time points during childhood (see Table 2). Exposure to life stress was assessed in each data collection wave, with a semistructured interview to the mother or both parents until the child age of 4 years, and thereafter self-reported by parents via questionnaire. The assessment evaluates adverse life events (about all relevant areas of life stress; e.g., death of close relative, parental divorce) in the preceding 1 year. Item used: Death of a parent.
7 Family relationship problems		Family relationship problems	
Problems with marriage relations	Reported at age 3 years. Question: Problems with marriage relations (yes/no) in the last 2 years. From questionnaire: Belangrijke levensgebeurtnissen [Important Life Events].		Family relationship problems by age 11 years. FAI item: low quality of partnership in two out of three areas (harmony, communication, emotional warmth). Assessed at multiple time points during childhood (see Table 2).
Unhealthy family functioning	General Functioning Subscale of the Family Assessment Device. Less healthy family functioning, assessed by parental report. Reported by mothers and partners at various time points during childhood (see Table 2).		

Supplementary Table 1. Description of childhood **adversities** in Generation R and MARS. (*continued*)

	GENR		MARS	
	Event	Description of event	Event	Description of event
8	Parental divorce/ separation	Parental divorce or separation ever occurring from child birth to age 9 years.	Parental divorce/ separation	Divorce/separation by age 11 years. MEL item (e.g. assessment at 11 years): Parental separation (>3 months)/divorce. Assessed at multiple time points during childhood (see Table 2).
9	Unemployment		Unemployment	
	Unemployment	Reported at age 3 years. Question: Unemployment (in the family) (yes/no) in the last 2 years. From questionnaire: Belangrijke levensgebeurtnissen [Important Life Events].		MEL item (e.g. assessment at 11 years): 1. Job loss, 2. Unemployment/unable to work (>3 months). Maternal and paternal unemployment assessed at multiple time points in childhood (see Table 2).
	Unemployment	Reported at age 9 years. Based on the Life Events Interview. Question: Involuntary unemployment of one of the parents (yes/no) occurring from childbirth to present.		
10	Physical abuse to child	Physical abuse by age 9 years. Based on the Life Events Interview. Question: Child was victim of physical violence occurring from childbirth to present.	Physical abuse to child	Assessed with the Childhood Trauma Questionnaire (CTQ). Retrospective assessment of sexual, physical, and emotional abuse, emotional and physical neglect during childhood and adolescence. The total scores for childhood sexual, physical, and emotional abuse were included in this study (see below). Cutoff points to define clinically significant abuse were based on previous studies (physical abuse = cut point of 8 or higher, sexual abuse = cut point of 8 or higher, emotional abuse = cut point of 10 or higher) (Bevilacqua et al., 2012; Walker et al., 1999). Score used: CTQ - physical abuse score.
11	Psychological abuse to child	Psychological abuse by age 9 years. Based on the Life Events Interview. Question: Someone almost used physical violence against the child and the child was scared. From childbirth to present.	Psychological abuse to child	CTQ - emotional abuse score

Supplementary Table 1. Description of childhood **adversities** in Generation R and MARS. (*continued*)

GENR		MARS	
Event	Description of event	Event	Description of event
12 Sexual abuse	Sexual abuse by age 9 years. Based on the Life Events Interview.	Sexual abuse	CTQ - sexual abuse score
Sexual comments or movements towards the child	Question: Someone made sexual comments or movements towards the child. From childbirth to present.		
Inappropriate sexual behavior	Question: Child experienced inappropriate sexual behavior. From childbirth to present.		

All references cited here are included in the manuscript.

Supplementary Table 2. Description of childhood **protective factors** in Generation R and MARS.

	Generation R Study			MARS		
	Protective factor	Age at assessment	Description	Protective factor	Age at assessment	Description
1	**Temperament**		Assessed with the Very Short Form of the Children's Behaviour Questionnaire (CBQ). Parents reported on the child reaction to events in the past 6 months. The CBQ includes three temperament dimensions: negative affectivity, surgency, and effortful control, assessed with sufficient internal consistency (Cronbach's alpha for negative affectivity: 0.75, surgency: 0.74, effortful control: 0.68; reported by Ghassabian et. al. (2014)). The three subscales were computed as the average of the respective questionnaire items in children who had less than 25% missing items per subscale. Higher scores mean greater negative affectivity, greater surgency and greater effortful control, respectively.	**Temperament**		Based on a parent interview and observations in two different days in both familiar and unfamiliar settings. A mean score was extracted from both interview and observations, and 9 temperament dimensions were assessed: activity, rhythmicity, approach/withdrawal, adaptability, threshold of responsiveness, intensity of reaction, the prevailing mood on a continuum between positive and negative mood, distractability/soothability, and attention span/persistence. Based on these dimensions, 2 factors were extracted: easy/difficult trait (related to mood, adaptability, etc.), and self-control (related to persistence, distractability, etc.) (for more information see: Pitzer et al., 2017). No inter-rater reliability was available due to organizational constrains, but assessments were based on highly structured protocols and all raters were carefully trained. Additionally, satisfactory inter-rater agreement was reported in a similar measure during infancy (κ = 0.82) (Pitzer et al., 2017). Higher levels in easy/difficult trait represent an easier temperament, and higher levels in self-control represent more self-control.
	Temperament - Negative affectivity	6 years	Negative affectivity subscale is defined by high positive loadings for sadness, fear, anger/frustration, and discomfort, and negative loadings from falling reactivity/soothability. This dimension is similar to neuroticism (Putnam and Rothbart et al., 2006).	Temperament - easy/difficult trait		
	Temperament - Surgency	6 years	Surgency/extraversion is characterized by impulsivity, high intensity pleasure and activity level, and negative loadings for shyness (Putnam and Rothbart et al., 2006).	Temperament - self-control	4.5 years	
	Temperament - Effortful control	6 years	Effortful control is characterized by high inhibitory control, attentional control, low intensity pleasure, and high perceptual sensitivity. This dimension is similar to conscientiousness/constraint.			

Supplementary Table 2. Description of childhood **protective factors** in Generation R and MARS. (*continued*)

	Generation R Study			MARS		
	Protective factor	Age at assessment	Description	Protective factor	Age at assessment	Description
2	**Child non-verbal IQ**	6 years	Assessed with two subtests of the non-verbal IQ test: SON-R 2.5-7. The reliabilities of the subtests scores used in Generation R were of 0.73 (Mosaics subtest) and 0.71 (Categories subtest) (Tellegen et. al., 1998).	**Child non-verbal IQ**	11 years	The Culture Fair Intelligence Test-20 (CFT-20) was used to assess non-verbal cognitive abilities (non-verbal IQ test)(Cattell, 1960)
3	**Child self-esteem**	9 years	We used an 18-item questionnaire based on the "CompetentieBelevingsSchaal voor Kinderen" (CBSK) questionnaire (Veerman et al., 1997), and the adapted question format by Wichstraum et al. (1995). The internal consistency was of ωc (categorical omega's) = 0.81 (de Lijster et. al., 2019). We computed a weighted total sum score when participants had data available for at least 13 items (allowing 28% missing values. Range of total score: 18-54). Higher scores represent greater self-esteem.	**Child self-concept**	8 years	Assessed with the German version of the Perceived Competence Scales (Harter & Pike, 1984) (German version by Asendorpf and Van Aken (1993)). Global self-concept was defined as the sum of the subscale scores: cognitive competencies, peer acceptance, and sports competencies. The subscales' internal consistency was between α = 0.58 (sports competencies) and 0.81 (peer acceptance) (Asendorpf and Van Aken, 1993; Dyer et al., 2007). Higher scores indicated greater self-concept.

Supplementary Table 2. Description of childhood **protective factors** in Generation R and MARS. (*continued*)

	Generation R Study			MARS		
	Protective factor	Age at assessment	Description	Protective factor	Age at assessment	Description
4	**Maternal sensitivity**	14 months	Maternal sensitivity was observed in a subgroup of children of Dutch national origin (with parents and grandparents born in the Netherlands) and it was based on two observed assessments: First, mothers and children participated in an 8-min psychophysiological assessment (child's electrocardiogram measurement while watching a Teletubbies episode (BBC/ Ragdoll Limited)), and then on a 5-min free play session in which the infant-mother interaction was unstructured. We used Ainsworth's rating subscales of ***cooperation*** and ***sensitivity***(Cooperation: the mother's ability to adjust her behavior in response to the infant activities, and to match cues from the infant. Sensitivity: the mother's ability to perceive and respond appropriately to the infant's signals). We calculated the maternal sensitivity score as the average of the standardized scores for both subscales in both assessments. This score was calculated for children who had at least one subscale score available, and higher scores indicated more sensitivity. The intercoder reliability (intraclass correlation coefficient) was 0.68 (Tharner et al., 2012).	**Maternal sensitivity**	3 months	Maternal sensitivity was assessed in a 10-minute semistructured session of nursing and playing situation ($\kappa > 0.83$) adapted from the categorical system for microanalysis of the early mother-child interaction (Jörg, et al., 1994, Holz, et al., 2018). Nine measures of mother-infant interaction behavior (e.g., vocalization, physical affect, variability) were created by coding behavior (present or absent) in 120 5s intervals using the Mannheim Rating System for Mother-Infant Interaction. **Maternal stimulation** (attempts to attract infant attention or establish contact (vocal, facial, or motor stimulation)) and **responsiveness** (behaviors in response to child's behaviors (vocal, facial, motor)) were coded based on the measures of mother-infant interaction. **Child** (vocal, facial, and motor) **responsiveness** was also coded. To compensate for differences in the mean between the three communication channels and to give equal weights to them, scores of vocal, facial, and motor responsiveness and stimulation were standardized and summed to create the respective **total score of maternal stimulation, responsiveness, and infant responsiveness. In these analyses, we used the measure of maternal stimulation (higher scores indicate more stimulation) to assess maternal sensitivity (based on previous evidence, e.g., Holz et al., 2018), and infant responsiveness was included as a covariate in analyses with maternal sensitivity.**

Supplementary Table 2. Description of childhood **protective factors** in Generation R and MARS. (*continued*)

	Generation R Study			MARS		
	Protective factor	Age at assessment	Description	Protective factor	Age at assessment	Description
	Additional cohort-specific measures					
5	**Friendship Quality**	9 years	Children's perceptions of the quality of their best friendship was assessed with a 10-item questionnaire based on the Friendship Quality Questionnaire. Items (e.g. "We give each other compliments") were rated on a 3-point Likert scale ("not true" to "very true") (Parker & Asher, 1993). The internal consistency was of ωc (categorical omega's) = 0.70 (de Lijster et. al., 2019). We computed a weighted total sum score when participants had data available for at least 8 items (allowing less than 25% missing values). Higher scores indicate a perception of greater friendship quality.	-	-	-

All references cited here are included in the manuscript.

Supplementary Table 3. Interaction between protective factors and childhood adversity on the **surface area** of the cortical regions of interest.

	Left ACC		Right ACC		Left medial OFC		Right medial OFC	
	ß (95%CI)	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**
Generation R Study								
Temperament - Negative affectivity, reversed	0 (-0.03; 0.03)	0.91	-0.01 (-0.05; 0.02)	0.48	-0.01 (-0.04; 0.03)	0.69	0 (-0.03; 0.03)	0.89
Temperament - Surgency	0 (-0.03; 0.04)	0.78	0.01 (-0.03; 0.04)	0.61	0 (-0.03; 0.03)	0.95	-0.01 (-0.04; 0.03)	0.71
Temperament - Effortful control	-0.01 (-0.04; 0.02)	0.45	0 (-0.03; 0.03)	0.99	-0.02 (-0.05; 0.01)	0.13	-0.01 (-0.04; 0.01)	0.34
Child non-verbal IQ	-0.03 (-0.06; 0)	0.09	-0.01 (-0.05; 0.02)	0.45	-0.01 (-0.04; 0.03)	0.69	-0.01 (-0.03; 0.02)	0.69
Child self-esteem	-0.01 (-0.04; 0.02)	0.38	0 (-0.03; 0.04)	0.76	0.01 (-0.02; 0.04)	0.38	0.01 (-0.01; 0.04)	0.31
Maternal sensitivity*	0.01 (-0.06; 0.09)	0.76	0.02 (-0.07; 0.10)	0.67	0.02 (-0.05; 0.09)	0.62	0.04 (-0.03; 0.12)	0.27
Friendship quality	-0.02 (-0.05; 0.01)	0.17	0 (-0.03; 0.04)	0.78	0 (-0.03; 0.03)	0.81	0 (-0.03; 0.02)	0.75
MARS								
Temperament								
Temperament - easy/difficult trait	0.08 (-0.03; 0.20)	0.16	0 (-0.13; 0.12)	0.98	-0.07 (-0.18; 0.04)	0.24	-0.03 (-0.14; 0.07)	0.53
Temperament - self-control	0.01 (-0.08; 0.11)	0.77	0.03 (-0.08; 0.13)	0.58	0.01 (-0.08; 0.10)	0.81	0.03 (-0.06; 0.11)	0.52
Child non-verbal IQ	-0.01 (-0.12; 0.10)	0.83	0.05 (-0.06; 0.17)	0.36	0.08 (-0.02; 0.18)	0.13	0.04 (-0.06; 0.14)	0.41
Child self-esteem	0.04 (-0.09; 0.17)	0.54	0.08 (-0.05; 0.22)	0.23	0.04 (-0.08; 0.17)	0.49	-0.02 (-0.13; 0.10)	0.78
Maternal stimulation (sensitivity)**	0.05 (-0.04; 0.14)	0.31	0 (-0.10; 0.10)	0.94	0.02 (-0.06; 0.11)	0.59	0.09 (0.01; 0.17)	0.03

Predictors included: cumulative adversity, protective factor (specific for each model), (child) sex, total intracranial volume, prenatal smoking, maternal national origin (only in Generation R), (child) age at the MRI scan (only in Generation R), obstetric risk, and the interaction term between each protective factor and cumulative adversity. Analyses with maternal sensitivity in MARS additionally adjusted for child responsiveness. Analyses with maternal sensitivity in Generation R not adjusted for national origin. Negative affectivity scores in Generation R were reversed.

All brain outcomes and adversity and protective factors were standardized. Abbreviations: ACC: Anterior cingulate cortex, OFC: Orbitofrontal cortex

Generation R N = 3,008. *Analyses with maternal sensitivity in N = 383.

MARS N = 179. **Analyses with maternal sensitivity in N = 173.

Supplementary Table 4. Interaction between protective factors and childhood adversities in relation to the **left and right** amygdala and hippocampus.

	Amygdala				*Hippocampus*			
	Left		Right		Left		Right	
	ß (95%CI)	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**
Generation R Study								
Temperament - Negative affectivity, reversed	0 (-0.04; 0.03)	0.89	0.01 (-0.02; 0.04)	0.63	-0.01 (-0.04; 0.02)	0.54	-0.01 (-0.04; 0.03)	0.64
Temperament - Surgency	0.02 (-0.02; 0.05)	0.35	0 (-0.03; 0.04)	0.81	-0.01 (-0.04; 0.02)	0.62	-0.01 (-0.04; 0.02)	0.51
Temperament - Effortful control	0.01 (-0.03; 0.04)	0.62	0 (-0.03; 0.03)	0.98	0.02 (-0.01; 0.05)	0.29	0.02 (-0.02; 0.05)	0.32
Child non-verbal IQ	-0.01 (-0.05; 0.02)	0.45	-0.02 (-0.05; 0.01)	0.24	-0.02 (-0.05; 0.02)	0.35	-0.02 (-0.05; 0.01)	0.28
Child self-esteem	0 (-0.03; 0.04)	0.79	0 (-0.04; 0.03)	0.89	0.01 (-0.02; 0.04)	0.66	0 (-0.03; 0.03)	0.86
Maternal sensitivity*	0.03 (-0.04; 0.11)	0.41	0.06 (-0.02; 0.14)	0.12	0 (-0.08; 0.07)	0.94	0.01 (-0.07; 0.09)	0.82
Friendship quality	-0.02 (-0.06; 0.01)	0.16	-0.02 (-0.05; 0.02)	0.31	-0.01 (-0.05; 0.02)	0.37	-0.01 (-0.05; 0.02)	0.44
MARS								
Temperament								
Temperament - easy/difficult trait	-0.04 (-0.15; 0.07)	0.49	-0.04 (-0.14; 0.07)	0.48	-0.04 (-0.16; 0.07)	0.48	-0.04 (-0.15; 0.08)	0.55
Temperament - self-control	0.02 (-0.07; 0.11)	0.64	-0.02 (-0.10; 0.07)	0.70	-0.01 (-0.11; 0.08)	0.79	-0.01 (-0.11; 0.09)	0.90
Child non-verbal IQ	-0.07 (-0.17; 0.03)	0.18	-0.09 (-0.18; 0)	0.06	-0.02 (-0.13; 0.08)	0.66	0.01 (-0.10; 0.12)	0.83
Child self-esteem	0.06 (-0.06; 0.18)	0.30	0.13 (0.02; 0.24)	0.02	0.08 (-0.04; 0.21)	0.18	0.08 (-0.04; 0.21)	0.19
Maternal stimulation (sensitivity)**	0.04 (-0.05; 0.13)	0.42	-0.01 (-0.09; 0.07)	0.79	0.03 (-0.06; 0.12)	0.53	0.03 (-0.06; 0.13)	0.48

Predictors included: cumulative adversity, protective factor (specific for each model), (child) sex, total intracranial volume, prenatal smoking, maternal national origin (only in Generation R), (child) age at the MRI scan (only in Generation R), obstetric risk, and the interaction term between each protective factor and cumulative adversity. Analyses with maternal sensitivity in MARS additionally adjusted for child responsiveness. Analyses with maternal sensitivity in Generation R not adjusted for national origin. Negative affectivity scores in Generation R were reversed.

All brain outcomes and adversity and protective factors were standardized.

Generation R N = 3,008. *Analyses with maternal sensitivity in N=383.

MARS N = 179. **Analyses with maternal sensitivity in N = 173.

Supplementary Table 5. Interaction between protective factors and childhood adversities in relation to brain outcomes in children **with mothers of European descent.**

	Global and regional brain outcomes						*Subcortical outcomes*			
	Cortical grey matter volume		Cerebral white matter volume		Cerebellar volume		Amygdala		Hippocampus	
	β (95%CI)	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**	**β (95%CI)**	**p-value**
Generation R Study										
Temperament - Negative affectivity, reversed	0 (-0.04; 0.04)	0.96	0.01 (-0.04; 0.05)	0.74	0.03 (-0.01; 0.08)	0.12	0.01 (-0.03; 0.04)	0.71	-0.01 (-0.04; 0.03)	0.79
Temperament - Surgency	0.02 (-0.02; 0.07)	0.37	0 (-0.04; 0.05)	0.93	0.03 (-0.01; 0.08)	0.15	0.02 (-0.02; 0.06)	0.33	0 (-0.04; 0.04)	0.98
Temperament - Effortful control	-0.02 (-0.06; 0.02)	0.32	0 (-0.04; 0.04)	0.90	0 (-0.04; 0.05)	0.88	0.01 (-0.02; 0.05)	0.47	0.02 (-0.02; 0.05)	0.41
Child non-verbal IQ	0.02 (-0.02; 0.06)	0.35	0 (-0.04; 0.04)	1.00	0 (-0.04; 0.05)	0.83	0 (-0.04; 0.04)	0.99	-0.02 (-0.06; 0.01)	0.23
Child self-esteem	0 (-0.04; 0.04)	0.99	0.01 (-0.03; 0.05)	0.63	0 (-0.04; 0.03)	0.82	0 (-0.04; 0.03)	0.88	0 (-0.04; 0.03)	0.84
Maternal sensitivity	-	-	-	-	-	-	-	-	-	-
Friendship quality	0 (-0.04; 0.04)	1.00	0.02 (-0.02; 0.06)	0.32	0.04 (-0.01; 0.08)	0.10	-0.02 (-0.06; 0.01)	0.21	-0.03 (-0.06; 0.01)	0.18

Note. Predictors included: cumulative adversity, protective factor (specific for each model), (child) sex, total intracranial volume (only in subcortical outcomes), prenatal smoking, (child) age at the MRI scan (only in Generation R), obstetric risk, and the interaction term between each protective factor and cumulative adversity. Analyses with maternal sensitivity in Generation R same as the main analyses. Negative affectivity scores in Generation R were reversed.
All brain outcomes and adversity and protective factors were standardized. Amygdala and hippocampus volumes are the mean volumes across left and right hemisphere.
Generation R N=1,947

Supplementary Table 6. Interaction between protective factors and childhood adversities in relation to the **volumes** of the cortical regions of interest in children **with mothers of European descent.**

	Left ACC		Right ACC		Left medial OFC		Right medial OFC	
	ß (95%CI)	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**	**ß (95%CI)**	**p-value**
Generation R Study								
Temperament - Negative affectivity, reversed	0 (-0.04; 0.05)	0.82	-0.03 (-0.08; 0.01)	0.15	-0.02 (-0.06; 0.01)	0.20	-0.02 (-0.05; 0.02)	0.44
Temperament - Surgency	0 (-0.05; 0.04)	0.91	0 (-0.04; 0.05)	0.85	0.01 (-0.03; 0.05)	0.71	0 (-0.04; 0.04)	0.96
Temperament - Effortful control	-0.01 (-0.04; 0.03)	0.70	-0.02 (-0.06; 0.03)	0.47	-0.01 (-0.04; 0.03)	0.69	-0.01 (-0.04; 0.03)	0.73
Child non-verbal IQ	0 (-0.04; 0.04)	0.84	0 (-0.04; 0.04)	0.94	0.01 (-0.03; 0.04)	0.71	0.01 (-0.03; 0.05)	0.53
Child self-esteem	-0.01 (-0.05; 0.03)	0.53	-0.01 (-0.05; 0.03)	0.72	0 (-0.03; 0.03)	1.00	0.02 (-0.01; 0.06)	0.18
Maternal sensitivity	-	-	-	-	-	-	-	-
Friendship quality	-0.02 (-0.06; 0.01)	0.20	0.01 (-0.04; 0.05)	0.73	0.02 (-0.02; 0.05)	0.39	0.02 (-0.02; 0.05)	0.37

Note. Predictors included: cumulative adversity, protective factor (specific for each model), (child) sex, total intracranial volume, prenatal smoking, (child) age at the MRI scan (only in Generation R), obstetric risk, and the interaction term between each protective factor and cumulative adversity. Analyses with maternal sensitivity in Generation R same as the main analyses. Negative affectivity scores in Generation R were reversed.
All brain outcomes and adversity and protective factors were standardized. Abbreviations: ACC: Anterior cingulate cortex, OFC: Orbitofrontal cortex
Generation R N=1,947

SUPPLEMENTARY METHODS

Participants

MARS

From the 309 participants included in the 25-year assessment, structural brain MRI data were collected in a subsample of 201. One participant with insufficient MRI data quality and one participant with systemic lupus erythematosus were excluded from analyses. Our final study sample consisted of 179 individuals who were right-handed and who had no current psychopathology, no use of psychotropic medication, and sufficient adversity data (Supplementary Figure 2).

Measures

Childhood adversity

When repeated measures of adverse events were available (e.g., information on family relationship problems collected at 3 months, 2, 4.5, 8, and 11 years in MARS), we combined the measures into one based on whether the event had ever occurred (or not) in childhood. This was performed because repeated measures were not available for all adverse events, thus impeding the counting of the number of occurrences. All adversities were dichotomized based on the occurrence of the event (yes/no), using thresholds established by the literature when needed (e.g., psychopathology symptoms (De Beurs, 2004)).

Adversities were, for the most part, prospectively reported by parents or caregivers. A few adverse events were retrospectively reported, such as childhood physical, psychological, and sexual abuse in MARS, which were self-reported by participants at age 23 years with the brief screening version of the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003). Despite the retrospective nature of this questionnaire, it was included in the adversity measure given the relevance of these adverse events.

Generation R.

Maternal marital status and whether the pregnancy was planned and/or wanted were self-reported via questionnaires during pregnancy. Maternal and paternal psychopathology were assessed at multiple time points during childhood using the depression and anxiety subscales of the Brief Symptom Inventory questionnaire (Derogatis, 1993). Poverty (yes/no) was defined based on the national low-income threshold in the Netherlands, adjusted with an equivalence factor to take into account the number of children and adults in the house and additionally adapted to the price changes over time (Centraal Bureau voor de Statistiek, 2008). Net income as well as the number of persons in

the household were reported via questionnaires during pregnancy. Information on the number of children was adapted from reports collected at child age 3 and 5 years.
At age 3 years, the main caregiver reported whether the family had experienced marital problems or unemployment in the preceding two years (yes/no). Family functioning was also assessed at child age 5 and 9 years with the General Functioning subscale of the Family Assessment Device (Byles et al., 1988; Epstein et al., 1983), and the resulting sum score was dichotomized based on established cut-offs to define unhealthy family functioning (Henrichs et al., 2010). Parental separation or divorce was based on maternal reports via questionnaires at child age 3, 5, and 9 years and was classified as ever versus never occurring during childhood, as described by Xerxa et al. (2020). Data on parental death, unemployment, and physical, psychological, and sexual abuse were collected via a Life Events interview with the main caregiver when children were 9 years old. This instrument evaluates the occurrence of multiple life events during the child's lifetime (Dunn et al., 2019), and it is based on the TRAILS study questionnaires (Amone-P'Olak et al., 2009) and the Life Events and Difficulty Schedule (Brown & Harris, 1978).

MARS.

Information on childhood adversities was primarily collected using three instruments. First, the Family Adversity Index is an assessment based on the enriched index defined by Rutter and Quinton (1977); it is described in more detail in Supplementary Table 1. Further information can be found in the study by Holz et al. (2016). Second, information on childhood adversities was also extracted from a shortened version of the Munich Events List (MEL) (Maier-Diewald et al., 1983). The assessment has been described in detail by Monninger et al. (2019). Third, physical, sexual, and psychological abuse were assessed with the brief screening version of the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003). The total score of each type of abuse was dichotomized based on previously defined cut-offs (Bevilacqua et al., 2012; Walker et al., 1999).

Protective factors

Child temperament was reported by the main caregiver in Generation R when children were 6 years old, based on the Very Short Form of the Children's Behavior Questionnaire (CBQ) (Putnam & Rothbart, 2006). This instrument assesses three dimensions of temperament: negative affectivity (reversed in our analyses to facilitate interpretation), surgency/extraversion, and effortful control (Ghassabian et al., 2014). In MARS, child temperament was based on a standardized parent interview and structured direct behavioral observations of the child in familiar and unfamiliar settings at child age 4.5 years (Pitzer et al., 2017), using rating scales and an interview approach adapted from Thomas et al. (1968). Two temperament factors were extracted from these data: the easy-difficult trait (mainly defined by loadings of distractability/soothability, mood, ap-

proach/withdrawal, and adaptability) and self-control (based on attention/persistence, distractability/soothability, and negative loadings of activity and intensity) (Pitzer et al., 2017).

Child self-esteem was reported by children at age 9 years in Generation R and at age 8 years in MARS. In Generation R, a Dutch version of Harter's Self-Perception Profile for Children (Veerman et al., 1997), with an adapted question format based on Wichstraum (1995), was administered. Global self-esteem was assessed as a weighted sum score of the 18 items of the questionnaire (see also: de Lijster et al. (2019)). In MARS, child self-concept was assessed using the German version of the Perceived Competence Scales (Harter & Pike, 1984) (German version by Asendorpf and Van Aken (1993)). This measure yielded information on cognitive competencies, peer acceptance, and sports competencies (Dyer et al., 2007); the sum of the subscale scores was included in the analyses as a global measure of self-concept, referred to as self-esteem throughout the study, for ease of comparability with Generation R.

Maternal sensitivity was assessed in both cohorts by direct observation of the mothers' behavior. In Generation R, maternal sensitivity was examined in a subsample of children of Dutch national origin (N = 383 in the pertinent analyses) during the 14-month laboratory visit. This measure was based on a stressful 8-minute psychophysiological assessment and on a 5-minute free play session, and was rated using Ainsworth's scales (Ainsworth et al., 1974; Tharner et al., 2012). In MARS, trained researchers observed the interaction between the mother and the 3-month-old infant during a 10-minute semi-structured nursing and playing session based on the categorical system for microanalysis of the early mother-child interaction (Holz et al., 2018; Jörg et al., 1994). Coding of this interaction is described in detail by Laucht et al. (2001). We included adequate maternal stimulation as a measure of maternal sensitivity given its potentially superior role in affecting the offspring's neurobiological and psychological development (Holz et al., 2018; Holz et al., 2021). Infant responsiveness was added as a covariate in these analyses to assess maternal behavior independent of the degree of child responsiveness (Holz et al., 2018).

Friendship quality was assessed at child age 9 years in Generation R. Children rated the quality of their best friendship based on an adapted version of the Friendship Quality Questionnaire (FQQ) (Parker & Asher, 1993). The ten items (e.g., *"we tell each other secrets"*) could be rated as "*not true*," "*somewhat true*," or "*very true*," and the total score range was 10–30 (de Lijster et al., 2019).

Brain morphology

The reconstructed brain images in Generation R and MARS were visually inspected for quality; images with inaccuracies or artefacts were excluded from analyses (Monninger et al., 2019; Muetzel et al., 2018; Muetzel et al., 2019).

Covariates

Generation R.

Information on child sex and birth weight was collected from hospital and midwife obstetric records. Prenatal smoking was self-reported by mothers during pregnancy and was categorized as "smoking during pregnancy" versus "never smoked during pregnancy." Maternal national origin was based on the country of birth of her parents and was defined as "European descent" (including Dutch, North American, European, and Oceanian participants) versus "Others" (e.g., Surinamese, Moroccan). Low birth weight (< 2.500 g) (Rogne et al., 2017) was included as a dichotomous measure (yes/no) of obstetric risk.

MARS.

Child sex, obstetric risk, and maternal smoking during pregnancy were assessed during a standardized interview with the parents at child age 3 months (Holz et al., 2014). Smoking was classified as "smoking during pregnancy" versus "never smoked in pregnancy." Obstetric risk was defined as a cumulative score of the presence of nine adversities during the perinatal period, as described by Laucht et al. (2000).

Statistical analyses - sensitivity analyses

Several sensitivity analyses were performed. First, we analyzed the interaction of childhood adversities with the protective factors on the surface area of the cortical regions of interest (left and right ACC and medial OFC). Second, considering the functional differences between the left and right amygdala (Sergerie et al., 2008) and that the developmental trajectory of the amygdala and hippocampal volumes has been described to differ in the left and right hemisphere (Uematsu et al., 2012), we examined whether the interaction between childhood adversity and the protective factors differed for the left and right amygdala and hippocampus. Third, the Generation R study is a multi-ethnic cohort, and the role and relevance of protective factors in the interaction with childhood adversity effects may differ across national origins due to cultural reasons (Choo et al., 2017). Hence, we repeated the interaction analyses between childhood adversity and the protective factors on brain morphology only in Generation R children with mothers of European descent.

Non-response analyses

In Generation R, we compared children included in our study sample (N = 3,008) to children who had childhood adversity data but no MRI scans available (N = 2,957). We used t-tests for continuous variables and chi-squared tests for categorical ones. There was no difference in the distribution of maternal national origin (European descent: study

sample: 66.0%, no MRI sample: 66.2%, p = 0.91) or child sex (study sample: 50.4% female, no MRI sample: 49.0% female, p = 0.29) between children included in the analyses and those with no MRI data. IQ scores were higher in children in the analyses (mean (SD) = 103.0 (14.9)) compared to those with no brain scans (mean (SD) = 100.3 (15.0), p < 0.001). The prevalence of early parenthood, psychological abuse, and physical abuse did not differ between the groups (p = 0.14, 0.80 and 0.23, respectively), whereas children without MRI scans available were more likely to be exposed to poverty than those included in the analyses (p = 0.003).

In MARS, we compared participants in the study sample (N = 179) with those who participated in the assessment wave in which MRI data were collected (25-year assessment) but had no MRI scans available (N = 108). We found no statistically significant difference in child sex (study sample: 58.7% female, no MRI sample: 51.9% female, p = 0.32), child IQ (mean (SD): study sample: 105.7 (11.2), no MRI sample: 104.1 (11.4), p = 0.25), prenatal maternal smoking (smoking during pregnancy: study sample: 22.3%, no MRI sample: 31.5%, p = 0.12), early parenthood (p = 0.53) and poverty (p = 0.51). Psychological abuse was more common in participants with no MRI scans available than in those included in the analyses (p = 0.01).

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9

General Discussion

GENERAL DISCUSSION

In the research presented in this thesis, we examined the child neurocognitive outcomes of early-life adversity and stress. The studies complement each other by addressing different adverse events and by implementing various operationalizations of the stress concept. Overall, we found no consistent evidence for a specific association of stress during pregnancy with child IQ and with preadolescent brain morphology. In contrast, a robust finding was observed for all adversities in childhood (i.e. cumulative childhood adversities, low-income, harsh parenting, and violence exposure): Childhood adverse events were associated with smaller global brain volumes, whereas associations with the limbic structures were only sometimes found or only a trend. In this chapter, I provide a global interpretation of the findings across studies without delving into the specifics of each particular study. I also discuss some of the methodological considerations that I deem highly relevant and I comment on their challenges and potential implications. To finalize, I outline the clinical relevance of the current studies series, and offer recommendations for future research.

What is Stress Exactly?

Over the course of this thesis, an invariable topic of discussion was the definition of stress. Reviewers, co-authors and readers repeatedly inquired about the definition of stress and what led us to deciding on the nature of the stress measure. So, what is stress exactly? There is no agreement over how to define stress (Schwarzer & Luszczynska, 2012). Whereas some define the stress exposure on the basis of occurrence of adverse events, i.e. "the negative environmental experiences that are likely to require significant adaptation…and that represent a deviation from the expectable environment" (McLaughlin et al., 2019), others focus on the individual psychological perception of stress and state that stress takes place when a person perceives that the demands from the environment are beyond their potential to adapt (Cohen et al., 2007; Pollak & Smith, 2021). Further, another relatively large group of researchers define the occurrence of stress as whether an event disturbs the homeostasis, and focus on the biological correlates of the stress exposure (Davis & Sandman, 2006).

These different approaches to the definition of stress (i.e. stress perception, adverse events, and biological correlates of stress) further translate into distinct measurements, offering complementary views on the stress exposure. Yet, the specific approach used to assess adversity and stress gains particular relevance in a series of cases. First, the assessment of *adverse events* may be the best measure possible when retrospectively collecting data on the stress exposure (although see below for a discussion about the specific challenges of measuring adverse events). For example, Jones et al. (2019) examined a cohort of 68 children whose mothers were exposed to a natural disaster, an ice

storm, when they were pregnant. Mothers were contacted *six months after the storm*, to examine the "objective stress" (e.g. "how many days were you without electricity? Were you ever in danger due to lack of food?"), and "subjective stress", a measure that enquired about post-traumatic stress disorder symptoms (King & Laplante, 2005). Not surprisingly, associations with child cognitive and linguistic functioning and with the child brain morphology were mostly, or even only, observed in relation to the "objective stress" measure (Jones et al., 2019; Laplante et al., 2008). As the authors themselves explain, the subjective stress measure could be affected by the time lapse. That is because a fair proportion of mothers that experienced high levels of acute stress during the storm may not have reported subjective stress at the moment of the data collection (Laplante et al., 2008) (think for example of women who were postpartum at the moment of data collection and not experiencing ice storm-related stress anymore), and thus, the effect of psychological stress related to the ice storm could have been clouded.

A second situation in which the type of stress assessment is relevant is in the study of non-severe adverse events. Compared to severe events (e.g. sexual abuse, the caregiver's death), adverse events that are more common in the community may vary in the individual interpretation (e.g. repeating a grade in school). In these cases, the assessment of the stress perception or the biological responses is more pertinent than the assessment of the event occurrence per se. This is because low-impact events do not present a significant hazard to mental health and could importantly underestimate the influence of childhood adversity on subsequent outcomes (Schilling et al., 2008). Finally, other very specific cases may render certain types of measurement less or more advantageous than others. For instance, when the outcome could be related to psychopathology (e.g. brain morphology) or if the outcome is a measure of psychopathology itself, an evaluation of the occurrence of adverse events may be more valid than the perception of stress, which could be affected by the reporter's mental health (Schwarzer & Luszczynska, 2012).

Importantly, some scholars argue that psychopathology symptoms such as anxiety and depression may be part of the stress concept. This perspective is built upon the fact that the perception of stress is not limited to the person's response to a single event, but it is also based on the chronic stressors, the personal environment and the individual's global mental well-being (Cohen et al., 1983; Kessler, 1979). After all, it is logical to expect that a person may find a specific event more stressful if, for example, in the preceding month or so, he or she was feeling that multiple difficulties were piling up and were not under control (Cohen et al., 1983). Building on this theoretical basis, stress perception is often conceptualized as an overarching construct that includes depression and anxiety *symptomatology* as well as stress per se (Gunnar & Doyle, 2020). For example, Kessler et al. (2002) developed a scale aimed to measure the non-specific psychological distress, and included questions such as: "During the last 30 days, how often did you feel so nervous that nothing could calm you down?". Similar questions asking whether the

person feels nervous and stressed/tense have been also included in other stress perception measures (Cohen et al., 1983), as well as in assessments of general symptoms of anxiety (different from generalized anxiety disorder) (Derogatis, 1993; Grant et al., 2008), clearly illustrating an overlap between the operational measures of psychopathology symptoms and stress perception. Furthermore, researchers have shown a high correlation between self-reported stress measures assessed during pregnancy and in the postpartum period with symptoms of depression and anxiety (Gunnar & Doyle, 2020).

Importantly, the optimal assessment of the less abstract "adverse events" is also far from being agreed upon. Which events should be assessed? Is this an adverse event or is it rather a risk factor for the occurrence of adverse events? In this discussion, I would like to focus on the second question, and I will start with bluntly stating that the answer may never become clear. Researchers, however, feel strongly about it, either in favor or against including a specific event as an adversity. This issue is particularly important for the exposure to low income (and poverty). To begin with, an infant growing up in a low-income family may not necessarily experience stress. Picture, for example, a young couple of parents who are pursuing higher education and who live with a very low salary or on government support. These parents may have strong social support and may themselves come from highly educated households, allowing the child to grow up in the presence of multiple potentially protective factors (see Pollak and Wolfe (2020) for an in-depth discussion on poverty, related family circumstances and their effect on children's neurodevelopment). In a different household, a family living with a similarly low income could be chronically exposed to poverty, to multiple adverse events and the child would have persistent cognitive stimulation deficits. Following this line of thinking, it is easy to understand why some researchers consider that low income may not be *per se* an adversity or a stress measure, but rather a risk factor for the occurrence of adverse events. Yet, it is often difficult to differentiate between low income and early-life adversity, because these often co-occur (D. Walsh et al., 2019). On the other hand, scholars that consider poverty as a stress measure or an adversity argue that it has an unquestionable impact on typical development, through several structural determinants of health in the society, such as health care access, working conditions, the house quality, accessibility to education, the environment in the community, and the child's nutrition (Marmot et al., 2008). I believe both approaches are valid and logical, and that at least in the case of low income and poverty, it may be at the same time both an adversity and a risk factor for (other) adverse events. In the current thesis, this was a long-standing point of discussion. Various approaches were taken in response. First, we examined the relationship of adversity and stress with the child outcomes, while controlling for socioeconomic status (SES) indicators. As described in Chapter 2, some associations that were initially found, such as a relation between prenatal stress and low IQ in Dutch children, were no longer observed after adjusting for SES indicators, suggesting that differences in IQ were not

per se related to the maternal experience of stress during pregnancy, but this association was rather a reflection of co-occurring differences in SES. This pattern was also partly observed in the relation between adverse events and child brain morphology. The findings described in Chapter 4 between the cumulative number of childhood adversities and the volume of several brain structures were attenuated, although not fully explained, by the adjustment for SES indicators. These results support the fact that differences in SES indicators co-occur with many adversities and stress exposure, and additionally suggest that the potential effect of low income and adverse events on child neurocognitive outcomes may share part of the mechanistic pathways, but not all. Therefore, whether low income is to be considered as an adverse event or a confounding factor should be carefully discussed for every study and decided based upon the specific research aims. In the study of prenatal stress and IQ, for example, we were interested in parsing out the confounding effect of SES, in order to assess whether the global perception of stress was associated with subsequent offspring outcomes, independent of societal differences in SES. In studies examining adverse events, and particularly including events that are often intertwined with poverty, like neighborhood safety or access to health care, it would be logical to assess poverty as an additional adversity.

Findings of the studies described in this Thesis

We examined maternal stress and exposure to adversity during pregnancy in three studies. First, as described in Chapter 2, prenatal maternal stress was not associated with child cognition in the majority of children. Second, the cumulative exposure to stressful adverse events in pregnancy was not associated with differences in the offspring brain morphology. Third, low family income in pregnancy was related with smaller amygdala volumes in children but this finding was not specific for pregnancy, as it was not statistically different from the amygdala volumes of children exposed to low family income only in childhood. These results were surprising and unexpected because evidence clearly supports the Developmental Origins of Health and Disease (DOHAD). The DOHAD paradigm states that exposures to adverse conditions during an early developmental period may have long-term consequences for health (Barker, 2007). This effect is generally explained by programming changes on regulatory systems that lead to developmental plastic modifications that determine subsequent health outcomes (Gunnar & Doyle, 2020). Explaining the biological mechanisms underlying the effect of prenatal stress on child neurodevelopment is challenging, because the effect would not be through direct exposure to adversity, but indirectly, through the effect of stress on the maternal biological functioning. First, hypothalamic-pituitary-adrenal (HPA) axis functioning and cortisol secretion are often believed to be a key pathway. Although studies in humans have not found strong support for a link between maternal prenatal stress reports and cortisol (Beijers et al., 2014), evidence from animal studies offers important insights.

Experiments in pregnant rats that have been adrenalectomized show that the induction of stress, while maintaining their glucocorticoid levels stable by exogenous administration, does not result in the offspring neurocognitive alterations that would normally occur after the exposure to stress, like learning deficits and increased anxiety (Gunnar & Doyle, 2020; Weinstock, 2011). Further, after mimicking the glucocorticoid levels of stress exposure, only some of the neurocognitive alterations appeared in the offspring, suggesting that glucocorticoids partly mediate the effect of stress in the offspring and that other mechanistic pathways are likely to be also involved (Gunnar & Doyle, 2020; Weinstock, 2011). Additional pathways often postulated include inflammation (Hantsoo et al., 2019), and increased vascular tone and reduced utero-placental blood flow that can lead to fetal oxidative stress and hypoxia (Bronson & Bale, 2016).

The literature on prenatal stress and child neurocognitive outcomes is inconsistent. Whereas some studies describe a relation between stressful life events in pregnancy and poorer child cognitive functioning, others do not find any association (for a recent systematic review see: Van den Bergh et al. (2020)). Among the many reasons, findings across studies may be inconsistent because of a difference in the confounding factors included, a prospective vs retrospective assessment of stress, and because the concept of stress varied considerably across studies, with some researchers addressing narrow stress definitions (e.g. stress related to the exposure to floods (Simcock et al., 2017)) while others implement a broad stress concept, including both stressful experiences and mood problems (depression and anxiety symptoms) (Gunnar & Doyle, 2020). The studies in this thesis offer complementary views with consistent results: although we assessed different measures of adversity and stress, little evidence for an association between prenatal stress and child neurodevelopmental outcomes was obtained across all studies that we performed. Why was this association not observed in our study sample? A potential explanation is related to the *severity* of adversity. The placenta inactivates around 80 to 90% of the maternal cortisol that enters the fetal circulation, thereby protecting the fetus from excessive cortisol levels (Rakers et al., 2020). In situations that substantially increase the maternal cortisol levels, more cortisol would cross the placenta barrier and generate a dysregulation of the fetal HPA axis (Rakers et al., 2020). Furthermore, cortisol levels have been related to offspring brain morphological differences (Buss et al., 2012), and at the cellular level, cortisol is known to influence the neuronal proliferation and differentiation (Anacker et al., 2013). Thus, I hypothesize that the levels of stress and uncertainty experienced by mothers in highly adverse conditions (e.g. severe natural disaster) rather than the stress levels in a community sample, may result in higher cortisol blood levels. Considering the findings of studies in this thesis, I postulate that, if prenatal stress has an effect on the child neurocognitive outcomes, only small effect sizes are to be found in population-based studies.

One association was consistently observed in most of the adversity and neuroimaging studies in this thesis: adversity was related to smaller global brain volumes, that is, the volumes of the total brain, the cortical grey matter and cerebral white matter (described in Chapter 3, 4, 5 and 6). This an interesting finding especially because the adverse events assessed in each study were not the same and data were collected at different time points. For example, smaller global brain volumes were found in children whose mothers reported harsh parenting behaviors at child age 3 years, as well as in children from Dutch families who were exposed to low income in early life (prenatal and/or early childhood), and also in relation to childhood adversities reported by mothers when children were 10 years old. In general, studies in children exposed to severe adversities support this finding. A narrative review described that maltreatment was related to reduced brain volumes in both the cortical gray and white matter (Bick & Nelson, 2016), and early-life poverty exposure was related to smaller brain volumes in a sample of 6-to-12 year-old children (Luby et al., 2013). Literature on the relation between adversities and smaller global brain volumes is generally based on small studies and clinical samples. Studies in this thesis extend the evidence to the general population using the Generation R cohort. Nevertheless, findings would benefit from replication in other population-based studies.

Methodological Considerations

Causality in the Association of Early-Life Adversity and Child Neurodevelopment

Now that I have discussed the evidence for an association between childhood adversity and smaller global brain volumes, the follow-up question is whether adversity has a causal effect on child brain morphology, or whether this association is explained by other factors. To assess the plausibility of causality, Sir Bradford Hill proposed nine criteria: strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment and analogy (Hill, 1965). While in the previous section, the strength, biological gradient and coherence were broadly described, I would like to present here a more detailed discussion of the temporality, consistency, experiment and plausibility criteria.

To begin with, a *temporal* relationship between exposure and outcome is generally considered a prerequisite for causation (Hill's criteria) (Glass et al., 2013). Whereas a temporal link has been found in animal studies (see previous section) (Gunnar & Doyle, 2020; Weinstock, 2011), this criteria is difficult to assess in humans, mainly because some adverse events are chronic or do not have a determinate period of exposure (e.g. psychological abuse). Furthermore, the possibility of a bidirectional association, or even reverse causality, between adversity and the neurocognitive outcomes is plausible. Considering

that psychopathology (e.g. attention deficit hyperactivity disorder (ADHD)) may have a potential bidirectional causal relation with childhood maltreatment (assessed with Mendelian randomization) (Warrier et al., 2021) and possibly also influence the likelihood of being exposed to bullying (Le et al., 2019); and that psychopathology and psychological traits ((Muetzel et al., 2018), including for example callous traits (Bolhuis et al., 2019), and ADHD (Hoogman et al., 2019)) are related to brain volumetric differences, it is possible that brain morphological differences precede in some cases the exposure to adversity.

Second, information, confounding and selection bias could underlie the relation of adversity and brain outcomes described in the literature. Overall, studies performed in different settings (e.g. population-based samples, samples of children with abuse reported to child protective services, institutionalized children, and individuals who developed psychiatric disorders post-exposure to adversity) have shown *consistent* results, thus supporting the robustness of a link between adversity and brain outcomes. This is because studies used different recruitment strategies, adversity definitions, measurement approaches, and are often affected by different confounding and selection factors (e.g. ethnic background, childhood depression). Yet, it is important to note that consistency in results does not imply causality, as studies could also be affected by the same unmeasured factors. To give an example, parental psychopathology and maladaptive psychological traits can be (as considered by some scholars) confounding factors, possibly being expressed in offspring brain morphology due to heritability components (Jansen et al., 2015; Smoller et al., 2019) and at the same time fostering a stressful environment for the child, through an impact on factors like parenting behavior and family functioning (Breaux et al., 2014). Additionally, child neurodevelopment (for example, the cognitive functioning and brain morphology) has the property of equifinality (as do most health-related outcomes). This concept refers to an outcome that can occur through multiple pathways or that has various contributing factors (Cicchetti & Rogosch, 1996), and implies that smaller brain volumes and lower cognitive function may result from adversity, but also from genetic variation (e.g. genes related to psychopathology) or additional events occurring in the same period as the adversity (e.g. different stressful events not assessed).

Third, *experimental* evidence for a causal relation between adversity and brain outcomes is limited and most studies are based on small sample sizes (Bonapersona et al., 2018). A causal effect of early-life adversity on the neural functioning of dopamine, a neurotransmitter associated with psychiatric diseases like schizophrenia, was shown in a meta-analysis of animal studies (Bonapersona et al., 2018). In humans, a study by Sheridan et al. (2012) examined children living in institutions who were randomly assigned to either go into foster care or remain in institutional care; and a sample of children who had never been institutionalized. Although there was no evidence for a difference in total grey and white matter volume between the two randomized groups, this study

was likely underpowered to detect a significant effect (Total N of children in randomized groups = 54) (Sheridan et al., 2012).

Finally, when discussing the *plausibility* of a causal effect, hypothesized mechanistic pathways also need to be considered. First, stunting has been proposed as an explanation, and in Chapter 6 we addressed this possibility. We found that poverty in Dutch children is related to smaller global brain volumes. The influence of early-life adversity, and especially of poverty, on global child growth is always considered as a potential explanation of brain differences because the association of poverty during childhood with smaller preadolescent brain volumes could reflect global stunting. Indeed, children exposed to poverty have been shown to have lower height (Mackenbach, 2006), and this could explain their smaller brain volumes. However, and contrary to what I expected, childhood height did not explain global brain volume differences in children exposed to early-life poverty, suggesting that the association observed specifically pertains to the child brain volume. Second, a direct effect of adversity on child brain morphology could be explained by neural plasticity, and there is evidence supporting this mechanism in animals. To give an example, neonatal maternal separation in rodents has been shown to reduce the dendritic length and the dendritic spine density in neurons from the prefrontal cortex and hippocampus (Monroy et al., 2010), and among others, the HPA axis is one biological pathway suggested to underlie the adversity influences on the neuronal cells. The HPA axis has a central role in the physiological response to stress (Lupien et al., 2009) and glucocorticoids may affect the neuronal development (Anacker et al., 2013). This pathway was demonstrated in rodents in relation to hippocampal morphology. Ivy et al. (2010) showed that stress promotes the secretion of CRH (corticotropin-releasing hormone) and by blocking the binding of CRH to its receptors in the brain, the effect of early-life stress on hippocampal anatomy and synaptic plasticity could be prevented. However, the evidence for HPA axis involvement in the stress effect (e.g. prenatal stress) in humans is not robust, and research has also shown that this is probably not the only pathway involved (Gunnar & Doyle, 2020; Weinstock, 2011). Other mechanisms likely implicated in the effect of adversity on brain morphological development are oxidative stress (Schiavone et al., 2013) and an alteration in the immune system (Danese & Lewis, 2017), and these have been reviewed in detail in Chapter 3.

Understanding whether the association between adversity and child brain morphology is causal and what are the intermediate mechanisms remains key to derive public health implications. Confirming causation would signal that there is a possibility of intervention (Glass et al., 2013), which would be guided by adversity studies, for example, into targeting specific time points and possibly also specific adverse events. Further, knowledge on the mechanisms of the adversity effects and the role of protective factors on the association between childhood adversity and brain morphology would allow the design of interventions that prevent or reduce the adversity consequences. Overall, this

growing literature, including the studies in this thesis, suggests that childhood, but not prenatal, adversity is associated with differences in brain morphology, and animal studies support an, at least partially, causal effect. This has, of course, important implications for future research, which are discussed at the end of this chapter.

Are Large Subcortical Limbic Volumes Always Better?

Researchers often assume that *larger* volumes of a brain structure represent *positive* outcomes or the presence of a *positive* influencing factor. Yet, this is not always the case, and I would like to discuss why taking as an example the limbic neuroanatomy and the infant-parent attachment relationship. Very early in life, infants develop an attachment bond with the parent, which is later encoded as the child's internal working model to deal with future stressful events (Groh et al., 2017; Van IJzendoorn et al., 1999). Following this line of thinking, it is thus understandable that the quality of attachment influences the developmental adaptation of the child, for example, through an effect on brain structures related to the stress response (Groh et al., 2017). The literature on the relation between infant-parent attachment and brain morphology is relatively novel, and one of the first studies was included in this thesis (Chapter 7). In our sample, we observed that disorganized attachment quality was related to a larger hippocampal volume, and the same pattern of association, although not significant, was observed for the amygdala volume. Both of these limbic structures are components of the stress-response system (Lupien et al., 2009). Similarly, larger limbic volumes have been described in relation to poor attachment quality in other studies (Lyons-Ruth et al., 2016; Moutsiana et al., 2015). Although the evidence is largely consistent, these findings seem counterintuitive at first sight, because larger volumes are generally expected to represent a better biological outcome or a better environment. In contrast, these results would imply that *smaller* amygdala and hippocampal volumes are related to a *better* quality of the infant-parent attachment relationship. One interpretation of these findings that I have not discussed before is that the *smaller* amygdala and hippocampal volumes observed in relation to *better* quality of attachment, are simply reflecting the presence of a protective factor. I propose this interpretation in retrospect, prompted by the evidence from the adversity studies included in this thesis. In all studies in which an adverse event was assessed, we found no association between adversity and the limbic structures or in some cases *smaller* volumes of these regions, although not always significant (Chapter 3, 5 and 6). This set of results strongly contrasts with the *smaller* limbic volumes that were observed in relation to *better* attachment. *Smaller* volumes of the limbic structures have also been described in relation to *greater* levels of *protective factors* like maternal sensitivity and parental nurturance (Bernier et al., 2019; Rao et al., 2010; Rifkin-Graboi et al., 2015). Although the latter evidence is not completely consistent, I propose that *smaller* limbic volumes may reflect a positive environment, i.e. the presence of protective factors,

and not always the outcome of a negative effect (additional thoughts on the relation between adversity, the protective factors and the limbic morphology are presented at the end of the Discussion section).

It is important, however, to note that the evidence is based on studies that used a single MRI assessment, effectively evaluating the limbic morphology at only one time point. The volume of both the amygdala and hippocampus follows a non-linear developmental trajectory, with a peak in preadolescence (Uematsu et al., 2012). Thus, analyses examining the limbic developmental trajectory using repeated MRI assessments may greatly increase our knowledge on the role of protective factors and the child neurodevelopment. A great example to illustrate this point is the study by Shaw et al. (2006), who demonstrated that the trajectory of cortical thickness *change* is a stronger neuroanatomical correlate of child intelligence than the cortical thickness measures at one time point. The relation of greater intelligence with a highly dynamic brain cortex would thus limit inferences on the brain morphology of intelligence from cross-sectional studies. Regarding infant-parent attachment, the large limbic volumes found in childhood and in early adulthood in relation to a poor attachment quality may suggest a different developmental trajectory (e.g. smaller growth rate, early maturation) of the subcortical limbic regions compared to that in children with a secure (or organized) attachment relationship. However, longitudinal MRI studies are needed to determine whether this is the case, as single MRI assessments do not accurately reflect brain changes over time (Kraemer et al., 2000).

Psychological Resilience and Where to Find It

Resilience refers to the relatively good mental health outcomes that some persons have, despite their exposure to adverse events (Rutter, 2006). The beauty of this notion is that it is more than a measure of mental well-being or social functioning. Resilience is a dynamic concept, in which adverse and protective factors interact to shape the individual's response (Rutter, 2006). Protective factors that could buffer the adversity effects (also known as "resilience factors" (Fritz et al., 2018) or "resilience-promoting factors" (Bonanno, 2021)) are usually grouped into person-centered factors, like self-esteem or temperament, and socio-contextual factors, like maternal sensitivity and social support (Bonanno & Mancini, 2008). Studying which factors foster resilience is crucial to help children who are exposed to early-life adversity.

Note, of course, that these protective factors may lead to positive outcomes per se, i.e. not only in the face of adversity, for example friendship strength is related to increased self-worth and decreased anxiety symptomatology in adulthood (Narr et al., 2019), and early-life IQ is related to adult academic achievement (Fagan et al., 2007). Protective factors like optimism, positive coping styles and caregiving support and sensitivity have also been found to be associated with brain structural differences, in particular with the

morphology of the orbitofrontal cortex (OFC), the anterior cingulate cortex (ACC), and the limbic subcortical regions (Dolcos et al., 2016; Holz et al., 2016; Kok et al., 2015; Luby et al., 2016). Interestingly, these regions have also been suggested to be related with early-life adversity (Holz et al., 2020), thus potentially representing converging points for the effect of adversity and the protective factors. However, the neurodevelopmental interaction between protective factors and adversity is not well understood, and the few studies on this topic were generally small and almost exclusively based on adult samples. I aimed to address this knowledge gap in Chapter 8 by using two birth cohort studies: the Generation R Study and the MARS. The main strength of analyzing adversity and protective factors in these cohorts is that both studies counted with data on similar adverse events and protective factors collected during childhood, and brain morphological measures were assessed in childhood in Generation R and in adulthood in MARS. Although the latter age difference did not allow a replication approach, it provided a complementary perspective on the results, based on the alignment of adversity and protective factors measures across both longitudinal cohorts. Interestingly, there was no consistent evidence for a buffering effect of the protective factors on the association between adversity and the volume of the brain regions outline above – most findings were small and only observed in one of the two cohort studies, thus suggesting that the structural brain correlates of psychological resilience are likely to be subtle or transient. Importantly, as previously summarized by Bonanno (2021), studies examining predictors of resilience find largely only modest effects, which is probably explained by inherently small effect sizes and by the high specificity of protective factors for each situational demand and point in time. I therefore consider essential that future studies: first, replicate our cohort-specific results using similarly large neurodevelopmental studies (Van IJzendoorn & Bakermans-Kranenburg, 2021); second, use repeated measures of brain morphology to explore whether the interaction of adversity and protective factors is associated with brain volumetric changes; and third, examine other brain outcomes not assessed here, like the limbic sub-regional volumes, and brain functional metrics. Finally, additional approaches to measure protective factors that account for their stability and their situational variation could prove useful in our ongoing quest for the neurobiological anatomy of resilience.

Latent Factors and Measurement Invariance

Latent variable modeling is a statistical method commonly used in psychology. The purpose of this approach is to understand the structure and the nature of abstract concepts that cannot be directly measured, like religiosity or stress perception (Beaujean, 2014). In order to assess these concepts, the researcher uses manifest variables (also known as indicator variables), which can be measured (e.g. by questionnaires) and reflect different aspects of the abstract concept (Beaujean, 2014). The use of latent factors allows

researchers to model constructs for which the specific *weights* and *relevance* of the indicators is not known or defined a priori, or for which the weights (and relevance) may vary across different populations (Milfont & Fischer, 2010). This latter, quite particular property of latent variable modelling is often overlooked by researchers, who inadvertently assume that a psychological construct has the same meaning across different groups, like men and women, or such as cultural and ethnic groups (Milfont & Fischer, 2010). Confirming psychometric equivalence of the concept across groups is important to be able to generalize results, and testing it helps to better understand the attributes of the construct itself (e.g. whether a specific characteristic plays a more relevant role in the definition of a latent construct in women compared to men). This property is known as measurement invariance, and can be achieved in various degrees, meaning that the latent construct does not need to be completely equivalent across groups in order to be comparable and generalizable across all groups involved (Beaujean, 2014). The first degree of invariance is the configural invariance, that is used to determine whether the structure of the model is the same across groups, which means that the number of factors and the pattern of loadings are equivalent across groups (Webber & Smokowski, 2018). In terms of our analyses, configural invariance would mean testing whether all stress indicators are associated with the stress latent factor across the different national origin groups. Second, we need to confirm that factor loadings are analogous across groups (metric invariance), which implies that the *strength* of the association between indicators and the latent construct is similar (Milfont & Fischer, 2010). In other words, if this degree of invariance is not met, the *meaning* of the stress construct is likely to be different across groups, such that, for example, financial instability or housing problems may be more relevant in the definition of stress (higher loadings) for an individual of African background compared to an individual of Dutch background, despite both having similar stress levels. Yet, to be able to confirm that the construct is comparable across groups, a third degree of invariance needs to be additionally achieved: the scalar invariance (or strong invariance), in which it is tested whether the intercepts of the indicator variables are the same across groups (Beaujean, 2014). If this final degree of invariance is not achieved, comparison across groups may lead to incorrect conclusions, because apparent mean differences in the latent factor across groups may simply reflect differences in the mean of indicator variables (Putnick & Bornstein, 2016). Applied to the context of stress, scalar non-invariance would mean that, for example, couple conflicts are *more common in one culture than in others*, irrespective of the stress latent factor levels (Putnick & Bornstein, 2016). In Chapter 2, I used a latent variable model to define the global stress construct using several aspects of the stress experience as indicators. Interestingly, we found that the latent factor was noninvariant across broad groups of national origins (metric invariance not achieved), whereas it had strong invariance in more narrowly defined groups. Although measurement invariance is mostly not tested

in studies on population-based cohorts like Generation R, our findings highlight the fact that the assessment of stress perception in a population needs to include the evaluation of whether there are differences in the understanding of stress, because the meaning ascribed to the stress concept by groups such as those from different national origins is not always the same, and could even differ based on further sub-classifications (e.g. Moroccan may be classified into Berber, Arabic or other origins, and Surinamese into Creole, Hindu or other origins) (e.g. Korevaar et al. (2013)).

Clinical Implications

It is difficult to extract direct clinical implications from the studies presented in this thesis. Yet, our findings offer insights on the long-term correlates of stress. Interestingly and consistently, we found no strong evidence for an association between prenatal stress and child neurocognitive outcomes. In contrast, postnatal adverse events were related to smaller global brain volumes in children. These results are relevant because in the general population prenatal and childhood stress and adverse events are experienced by a nontrivial proportion of individuals. Percentages depend on the specific measures assessed, but vary between 17 to 63% for stress and adversity during pregnancy (Salm Ward et al., 2017; K. Walsh et al., 2019) and may amount to 50% in childhood (Child and Adolescent Health Measurement Initiative, 2018-2019; McLaughlin et al., 2019). In our sample, these percentages were of 36% and 35%, respectively. Regarding prenatal stress and adversity, finding no long-term link with the neurocognitive outcomes does not rule out an effect of prenatal stress on brain morphology and cognitive functioning, but suggests that the long-term associations of prenatal stress with IQ, global or limbic brain volumes likely have only small effect sizes in children from the general population of highly-developed industrialized countries (WEIRD societies (Henrich et al., 2010)). Knowing that prenatal stress, defined broadly, is probably only weakly related to child IQ and the volume of several different brain structures may offer reassurance to parents, and may even reduce the pregnancy-related stress. Regarding adversity during childhood, we show that even in children from average families, the experience of adverse events is related to neuroanatomical differences, and that the accumulation of multiple events plays an important role.

Further, we did not find evidence for a strong buffering effect of several childhood protective factors on the link between adversity and brain structure, providing a preliminary perspective on this interaction effect. More research directed to the identification of factors that moderate the adversity-neurocognitive outcomes relationship remains of uttermost importance, given that not all children who are exposed to early-life adversity develop psychological and cognitive problems later in life (Smith & Pollak, 2020).

While the primary objective of studies examining childhood adversity and neurocognitive outcomes is the establishment of clinically effective interventions that limit

the effects of adversity on child development, it is too early to generate evidence-based interventions. The literature on prenatal adversities is scarce and the quest for elucidating the mechanisms of the potential childhood adversities effects is still ongoing (Pollak & Smith, 2021). Both our findings with the prenatal and postnatal stress measures could aid to redirect the focus of future research. On the one hand, our results suggest that studies aiming to assess the impact of early-life stress on brain morphology in the general population should examine the mechanisms underlying the association observed for childhood stress (See also the Future research section). On the other hand, the lack of robust evidence for an association of prenatal stress with neurocognitive outcomes extends what is currently known and emphasizes the need for simultaneous evaluation of the role of adverse events, psychological stress perception and biological measures of stress (like cortisol) using similarly large study samples.

Future research and some (among the many) final considerations

The studies in this thesis add to the existing literature by demonstrating that the association between childhood adversities and brain morphology is robust and present even in children from the general population. Importantly, research suggests that this association is partly causal. So what should we investigate next? First, I recommend future studies to include multiple measures of stress in the same study (i.e. biological measures of stress (e.g. HPA axis and immune system measures), (child's) individual perception of stress and assessment of the adverse events). This will help us gain insight on whether particular measures of stress are more relevant to the child brain development and how these measures are related in different contexts. Specifically, measures on child perception of stress are currently lacking and I agree with Smith and Pollak (2020) in that incorporating them in future research would greatly advance our understanding of the adversity effects. Second, longitudinal studies with repeated assessments of adversity and brain morphology could help to establish the extent of a bidirectional effect and offer insights on the relation between adversity and brain volumetric *changes*. Third, we did not find robust evidence for a moderating effect of protective factors on the relation between early-life adversity and brain morphology, but these results are far from conclusive. Although it is possible that resilience is not directly related to neuroanatomical differences, further moderating factors, brain outcomes and replicating studies should be the goal of future research. After all, it is essential to identify factors that buffer the adversity effects, to understand how and in whom should we intervene to promote resilience after the exposure to early life stress.

I finalize this thesis by addressing a question that posits a major scientific challenge: Smaller limbic volumes (amygdala and/or hippocampus) have been reported by studies of children's neurodevelopmental outcomes (including some of this thesis) in relation to both: a protective factor (good quality of attachment, or parent support) and early-life

adversity. How is this possible? For a start, it is important to be aware that the evidence for both exposures is far from conclusive (see Chapter 3 and 7 for details). Further, findings seem to be somewhat puzzling, with no association observed in relatively large studies (e.g. Chapter 4) and in meta-analyses about severe adversities (e.g. no hippocampal difference in children exposed to maltreatment (Riem et al., 2015)). One explanation could be the timing and duration of the exposure. In fact, acute and chronic stress have been shown to lead to different physiological responses, for example, with an immune response characterized by catecholamines and glucocorticoids (high cortisol levels) if the stress is acute, and with immune suppression and reduction of cortisol levels if the stress is chronic (McEwen, 2017; Miller et al., 2007). A second explanation could be related to the fact that the influence of protective factors, as well as the effect of early-life adversity, may be specific for subfields of the amygdala and hippocampus. Recent studies have started examining the limbic subfields (see for example: Malhi et al. (2019)), but much more remains to be explored.

The studies included in this thesis contribute with thoughtful and novel evidence to this research question. We used multiple measurement approaches to early-life stress in the general population, we accounted for key confounding factors, and addressed the role of infant-parent attachment in a uniquely large pediatric sample. Our findings contribute with a preliminary view on the relation between adversity, protective factors and various neurocognitive outcomes in children from the general population.

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Summary / Samenvatting

SUMMARY

The literature background and aims of this thesis are described in **Chapter 1.** Fetal life and childhood are characterized by dramatic brain changes that start with the differentiation of the neural tissue, and continue with the formation of neurons and synapses, neural migration and myelination. During this period, adverse, but also positive, environmental factors may greatly influence brain typical growth and may have long-lasting consequences. The studies in this thesis were performed to address three main research gaps. First, the association between prenatal stress and child neurocognitive outcomes was not well-known. Previous studies were mostly based on data that was collected retrospectively, which could distort the appraisal of the stress experience. Second, evidence on childhood adversities and brain morphology was limited by studies largely based on small samples and participants exposed to *severe* adversities only. Third, research on brain structural correlates of resilience was and still is in its early stages, and studies mainly used cross-sectional assessments. This thesis is the compilation of studies that assessed stress and adversity using different approaches, and investigated the relation between adversities and early-life stress with subsequent neurocognitive outcomes in the general population. This thesis also includes studies that addressed the role of protective factors, and the interplay between adversity and multiple protective factors in relation to brain morphology. Data from the population-based Generation R Study, and the high-risk Mannheim Study of Children at Risk were used in the studies presented here.

Section A includes studies on the relation between adverse events and early-life stress with the child neurocognitive outcomes. In **Chapter 2**, we examined prenatal maternal stress modelled as a broad latent construct, which was based on multiple indicators of the stress exposure. We investigated whether prenatal stress was associated with child non-verbal cognition at age 6 years, and contrary to our expectations, we found very little evidence for this link, with only a small association observed in the Moroccan/Turkish minority group. Importantly, this study allowed us to test and describe the measurement invariance of the stress latent construct across national origin groups, and results suggested that there were some differences in the meaning attributed to the concept of stress across groups. In **Chapter 3**, we examined harsh parenting, independently reported by mothers and fathers, in relation to brain morphology at age 10 years. Maternal harsh parenting was found to be associated with smaller total gray, cerebral white matter and amygdala volumes, but not with the hippocampus or the white matter microstructural metrics. Interestingly, in this study there were similar associations, although not significant, for paternal harsh parenting. In **Chapter 4**, we used a different approach to early-life stress. We modelled the *cumulative exposure* to adverse events during the prenatal and childhood periods. This study showed no strong evidence for an

association between prenatal adversities and child brain volumes, and also no relation with offspring head circumference in the third trimester of pregnancy. Contrastingly, cumulative exposure to adversity during childhood was robustly related to differences in grey and white matter volumes at age 10 years, but not to the amygdala or hippocampal volumes. In **Chapter 5,** we examined whether two adverse events ("physical attack", and "threatened violence") often included in the "adversity – threatening experiences" classification would have a similar relation with child brain morphology. Contrary to what we hypothesized, physical attack during childhood, but not threatening violence, was associated with smaller global brain volumes. Finally, we focused on poverty in **Chapter 6**. Although considered by some researchers as an adversity, poverty is defined by others as an environmental factor equally relevant but different from adversity. In our study, we examined the exposure to low income during pregnancy and childhood, and we found that children from the Dutch majority group had smaller global brain volumes when exposed to poverty in early life, compared to non-exposed Dutch children. Interestingly, this finding mediated the relation between low-income and poor school performance. Overall, studies from **Section A** support an association between *childhood* adversity, independent of the type of measurement, with child brain morphology, but little-to-no evidence for long-lasting neurocognitive outcomes of *prenatal* stress.

In **Section B**, we addressed the role of protective factors. In **Chapter 7**, we made use of a uniquely large observational dataset, part of the Generation R cohort. We performed this study in a subsample of 551 children, in whom the quality of infant-parent attachment was assessed with the Strange-Situation Procedure and brain morphological measures were collected at age 10 years. We showed that children with an organized infant attachment pattern had smaller hippocampal volumes compared to those with a disorganized attachment. Importantly, this was observed in both hemispheres and was robust to adjustment for confounders. This finding was surprisingly consistent with previous studies of attachment, maternal sensitivity and measures of parental nurturance, suggesting that *smaller* limbic volumes could be related to a *positive* environmental factor. Building on the evidence collected in the preceding chapters, we examined in **Chapter 8** whether protective factors during childhood modified the association between childhood adversity and brain morphology. We performed these analyses with a neurodevelopmental approach, using two longitudinal birth study cohorts, the Generation R Study, in which brain measures were collected in childhood, and MARS, with brain measures at age 25 years. These two cohorts were selected to address whether the interplay of adversity and protective factors was similar across different settings. However, we found little evidence for robust interaction effects between adversity and multiple protective factors on the brain regions of interest: the amygdala, hippocampus, anterior cingulate cortex, medial orbitofrontal cortex and cerebellum. These findings may suggest that the brain volumetric correlates of resilience are likely subtle and not

consistent across different contexts. Future studies may assess this interaction effect using repeated measures of adversity and brain morphology, and examine also other brain outcomes not explored here.

To conclude, **Chapter 9** includes a general discussion of the findings, in which I provide a global interpretation of the studies, discuss the main methodological implications, and outline the clinical relevance and recommendations for future research.

SAMENVATTING

De achtergrond en doelstelling van dit proefschrift zijn beschreven in **Hoofdstuk 1**. Tijdens het foetale leven en de kindertijd vinden er grote veranderingen plaats in het brein, beginnend met de differentiatie van het neurale weefsel en gevolgd door de vorming van neuronen en synapsen, neurale migratie en myelinisatie. Tijdens deze periode zouden nadelige, maar ook positieve, omgevingsfactoren van grote invloed kunnen zijn op de groei van het brein en daarmee langdurige gevolgen kunnen hebben. De studies beschreven in dit proefschrift werden uitgevoerd om drie hoofdvragen te onderzoeken. Ten eerste was er nog onvoldoende bekend over de associatie tussen prenatale stress en neurocognitieve uitkomsten bij het kind. Eerdere studies waren vooral gebaseerd op retrospectief verzamelde data, wat de beoordeling van stressbeleving zou kunnen vertekenen. Ten tweede was het onderzoek naar moeilijkheden in de kindertijd en morfologie van het brein beperkt tot studies met kleine studiepopulaties en deelnemers blootgesteld aan alleen *ernstige* traumatische ervaringen, zoals mishandeling of misbruik. Ten derde, onderzoek naar structurele brein-associaties van veerkracht was en is nog steeds in de beginfase. Daarnaast waren deze studies vooral gebaseerd op cross-sectionele assessments. Dit proefschrift is een compilatie van studies naar stress en traumatische ervaringen op verschillende manieren, en onderzocht te relatie tussen traumatische ervaringen en stress in het vroege leven met neurocognitieve uitkomsten onder de algemene bevolking. Dit proefschrift bevat ook studies gericht op de rol van beschermende factoren en de wisselwerking tussen traumatische ervaringen en verschillende beschermende factoren in relatie tot brein morfologie. Data van de populatie-gebaseerde Generation R Studie en de hoog-risico Mannheim Study of Children at Risk zijn gebuikt in de studies beschreven in dit proefschrift.

Sectie A bevat studies naar de relatie tussen negatieve levensgebeurtenissen en stress in het vroege leven met neurocognitieve uitkomsten bij kinderen. In **Hoofdstuk 2** onderzochten we maternale prenatale stress gemodelleerd als een breed latent construct gebaseerd op verschillende indicatoren van bloostelling aan stress. We onderzochten of prenatale stress geassocieerd was met non-verbale cognitie bij kinderen van 6 jaar oud. In tegenstelling tot onze verwachtingen vonden we, afgezien van een zwakke associatie in de Marokkaans/Turkse minderheidsgroep, weinig bewijs voor deze relatie. Belangrijk is dat deze studie ons in staat gesteld heeft om de "measurement invariance" van het stress latent construct tussen etnische groepen te testen en te beschrijven. De resultaten hiervan wijzen erop dat er wat verschillen zijn in de betekenis toegekend aan het concept stress tussen etnische groepen. In **Hoofdstuk 3** onderzochten we hardhandige opvoeding, gerapporteerd door moeders en vaders onafhankelijk van elkaar, in relatie tot morfologie van het brein op de leeftijd van 10 jaar. Hardhandige opvoeding door de moeder was geassocieerd met lagere totale cerebrale grijze-, witte stof en amygdala volu-

mes, maar niet met de hippocampus of de witte stof microstructurele maten. Interessant is dat er in deze studie ook soortgelijke associaties gevonden werden voor hardhandige opvoeding door de vader, hoewel deze niet statistisch significant waren. In **Hoofdstuk 4** gebruikten we een andere methode om stress in het vroege leven te onderzoeken. We modelleerden de *cumulatieve blootstelling* aan negatieve levensgebeurtenissen tijdens de prenatale periode en de kindertijd. In deze studie vonden we geen sterk bewijs voor een associatie tussen prenatale negatieve levensgebeurtenissen en brein volumes bij kinderen, en ook geen relatie met de hoofdomtrek van het kind in het derde trimester van de zwangerschap. Daarentegen was cumulatieve bloostelling aan negatieve levensgebeurtenissen tijdens de kindertijd robust gerelateerd aan verschillen in grijze en witte stof volumes op de leeftijd van 10 jaar, maar niet aan amygdala of hippocampus volumes. In **Hoofdstuk 5** onderzochten we of twee nadelige levensgebeurtenissen ("fysieke agressie" en "dreiging met geweld"), vaak geschaard onder de "traumatische ervaringen – bedreigende ervaringen" classificatie , een soortgelijke relatie hebben met brein morfologie van het kind. In tegenstelling tot onze hypothese was slachtoffer van fysieke agressie tijdens de kindertijd, maar niet dreiging met geweld, geassocieerd met kleinere globale brein volumes. Als laatste hebben we ons in **Hoofdstuk 6** gericht op armoede. Hoewel sommige onderzoekers armoede als een negatieve levensgebeurtenis/traumatische ervaring beschouwen, beschouwen andere onderzoekers armoede als een omgevingsfactor die even belangrijk is, maar anders dan een moeilijkheid. In onze studie onderzochten we de blootstelling aan laag inkomen tijdens de zwangerschap en in de kindertijd en vonden dat kinderen uit de Nederlandse meerderheidsgroep kleinere globale hersenvolumes hadden wanneer zij waren blootgesteld aan armoede vroeg in het leven, vergeleken met Nederlandse kinderen die niet waren blootgesteld aan armoede. Interessant is dat deze bevinding een mediator was in de relatie tussen laag inkomen en slechte schoolprestaties. Overall ondersteunen de studies uit **Sectie A** een associatie tussen traumatische ervaring *in de kindertijd,* onafhankelijk van het type meting, en brein morfologie in de kindertijd, met weinig tot geen bewijs voor langdurige neurocognitieve uitkomsten van prenatale stress.

In **Sectie B** onderzochten we de rol van beschermende factoren. In **Hoofdstuk 7** gebruikten van een uitzonderlijk grote observationele dataset van het Generation R cohort. We voerden deze studie uit in een subpopulatie van 551 kinderen voor wie de kwaliteit van hechting tussen peuter en ouders onderzocht was met de "Strange-Situation Procedure" en er metingen van de morfologie van het brein gedaan waren op 10-jarige leeftijd. We toonden aan dat kinderen met een georganiseerd hechtingspatroon kleinere hippocampus volumes hadden vergeleken met kinderen met een ongeorganiseerd hechtingspatroon. Belangrijk is dat deze associaties aanwezig waren voor beide hemisferen en bestand waren tegen correctie voor confounders. Deze bevinding was consistent met de bevindingen van eerdere studies naar hechting, moederlijke sensitivi-

teit, en moederlijke koestering, suggererend dat *kleinere* limbische volumes gerelateerd zouden kunnen zijn aan een *positieve* omgevingsfactor. Voortbordurend op het bewijs uit voorgaande hoofdstukken onderzochten we in **Hoofdstuk 8** of beschermende factoren tijdens de kindertijd een effect modificator was in de relatie tussen moeilijkheden in de kindertijd en brein morfologie. We deden deze analyses vanuit het perspectief van de neurologische ontwikkeling, gebruikmakend van twee longitudinale geboortecohorten, de Generation R Studie, met metingen van het brein in de kindertijd en MARS, met metingen van het brein op 25-jarige leeftijd. Deze cohorten werden geselecteerd om te onderzoeken of de wisselwerking tussen traumatische ervaring en beschermde factoren gelijk was in verschillende settings. We vonden echter weinig bewijs voor robuste interactie effecten tussen moeilijkheden en verschillende beschermende factoren op de delen van het brein van belang voor ons onderzoek: de amygdala, hippocampus, cortex cingularis anterior, cortex orbitofrontalis medialis en het cerebellum. Deze bevindingen zouden kunnen suggereren dat structurele veranderingen in het brein gecorreleerd aan veerkracht subtiel zijn en niet consistent tussen verschillende contexten. Toekomstige studies zouden dit interactie-effect kunnen onderzoeken door middel van herhaalde metingen van moeilijkheden en brein morfologie. Daarnaast zouden zij zich kunnen richten op andere brein uitkomsten die niet onderzocht zijn in dit proefschrift.

Als afsluiting bevat **Hoofdstuk 9** een algemene discussie van de bevindingen, waarin ik een globale interpretatie geef van de studies, de belangrijkste methodologische implicaties bediscussieer en de klinische relevantie en aanbevelingen voor verder onderzoek beschrijf.

A

Addendum

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PHD PORTFOLIO

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PhD period: 2016 – 2021
Promotors: Prof. Dr. H. Tiemeier
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Prof. Dr. M. J. Bakermans-Kranenburg
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	Year	ECTS
1. PhD training Master of Science in Health Sciences, Specialization in Epidemiology Netherlands Institute for Health Sciences (NIHES), the Netherlands		
Required courses		
Principles of Research in Medicine (ESP01)	2016	0.7
Introduction to Global Public Health (ESP41)	2016	0.7
Methods of Public Health Research (ESP11)	2016	0.7
Fundamentals of Medical Decision Making (ESP70)	2016	0.7
Primary and Secondary Prevention Research (ESP45)	2016	0.7
Social Epidemiology (ESP 61)	2016	0.7
Study Design (CC01)	2016	4.3
Biostatistical Methods I: Basic Principles (CC02)	2016	5.7
Clinical Epidemiology (CE02)	2016	5.7
English Language (SC01)	Exempted	1.4
Biostatistical Methods II: Classical Regression Models (EP03)	2017	4.3
Principles in Causal Inference (EP01)	2018	1.4
Introduction to Medical Writing (SC02)	Exempted	2
Advanced elective courses, NIHES		
Repeated Measurements in Clinical studies (CE08)	2017	9
Principles of Epidemiologic Data-analysis (EWP25)	2017	0.7
Courses for the Quantitative Researcher (SC17)	2017	1.4
Psychiatric Epidemiology (EP12)	2017	1.1
Topics in Meta-analysis (ESP15)	2017	0.7
Principles of Genetic Epidemiology (ESP43)	2017	0.7
Causal Mediation Analysis (ESP69)	2017	0.7
Causal Inference (ESP48)	2017	1.4

Skill Courses			
	Scientific Integrity course, Erasmus MC	2017	0.3
	FreeSurfer Course, Universitat Pompeu Fabra, Barcelona, Spain	2017	1.0
	Safety Training MR Personnel, Level 1 & 2	2018	0.1
2.	**Symposia, Conferences & Workshops**		
	The "Santander Summer School 2017: Social Processes and Mental Health", Heidelberg, Germany (oral presentation)	2017	2.2
	ISRCAP Scientific Meeting 2017, Amsterdam, the Netherlands (attendance)	2017	1.1
	Stress-NL consortium meeting 2017, Amsterdam, the Netherlands (attendance)	2017	0.3
	Imaging Research on the Move Meeting, Erasmus MC (short oral presentation)	2018	0.1
	SRCD 2019 Biennial Meeting, Baltimore, United States (Poster and oral presentation)	2019	0.8
	PACE Consortium Meeting, Erasmus MC (short oral presentation)	2019	0.3
	26th Annual Meeting of the Organization for Human Brain Mapping, virtual (attendance)	2020	1.3
	8th Annual Flux Virtual Congress, virtual (poster presentation)	2020	0.8
	AACAP's 2020 Virtual Annual Meeting, virtual (poster presentation)	2020	1.7
3.	**Teaching activities**		
		Year	**ECTS**
	Minor research project of bachelor student: Lorenza Dall'Aglio *Correlates of bullying behaviour in children from the general population*	2016-2017	2.0
	Master thesis: Puck Weve *Cheating behaviour in children as a predictor of anxiety – The Generation R Study*	2017-2018	2.0
	Daily supervision of project by visiting PhD student: Yuna Koyama *Poverty from fetal life onward and child brain morphology: differential association by minority status*	2019-2020	2.0
4.	**Other activities**		
	Data collection - Generation R general tasks	2016-2020	
5.	**Grants and Awards**		
	Full Scholarship for "the Santander Summer School 2017: Social Processes and Mental Health". Heidelberg, Germany	2017	
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1 ECTS (European Credit Transfer System) is equal to a workload of 28 hours

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