Neural and Behavioral Correlates of Childhood Social Exposures

NEURAL AND BEHAVIORAL CORRELATES OF CHILDHOOD SOCIAL EXPOSURES

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Abstract

Childhood behavior problems substantially impact individuals, families, and communities. Poverty and social disadvantage are known risk factors for child behavior problems, while healthy social environments may buffer effects of social disadvantage. Yet, neural mechanisms underlying these relationships remain understudied. This dissertation presents three studies about neural and behavioral correlates of childhood social exposures using data from the Generation R Study, a birth cohort in Rotterdam, the Netherlands.

The first study (n = 2,653) investigates the association between one facet of a healthy childhood social environment—healthy family functioning—and brain white matter microstructure in preadolescence. The study finds evidence that healthier levels of maternal-report prenatal family functioning, but not family functioning in mid-childhood, are associated with lower mean diffusivity across the brain, a marker of more favorable white matter microstructure.

The second study (n = 2,905) assesses two closely related types of childhood physically threatening experiences—actual violence exposure and mere threatened violence exposure alone—and their association with both preadolescent global brain structure and the structure of specific corticolimbic regions of the brain involved in threat response. Results suggest actual violence exposure—but not mere threatened violence exposure alone—is associated with smaller global cortical gray matter, subcortical gray matter, and white matter volume, even after extensive adjustment for possible confounders. We also find that actual violence exposure is associated with smaller amygdala volume.

The final study (n = 3,154) investigates mediation and moderation of the association between violence exposure and behavior problems. We find that childhood violence exposure is associated with higher total preadolescent behavior problems and lower amygdala volume on average. We also find that healthy family functioning—but not high parental socioeconomic status or sex—may substantially alter both of these associations. For example, violence exposure among children of lower functioning families is associated with more preadolescent behavior problems than among children of higher functioning families. However, we find no evidence that amygdala volume partially mediates the association between violence exposure and behavior problems.

Together, these studies contribute to research investigating brain-based mechanisms linking childhood risk and protective factors to the development of childhood behavior problems.

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Introduction

Childhood mental disorders, including behavior problems, impose significant short- and long-term impacts on society. They account for more medical spending ($13.9 billion in 2012) on children than any other condition, and they have substantial impact on individuals and their families as well as their communities.[1,2] For example, behavior problems in children are linked to a range of other health-related problems later in life, including smoking, substance use disorder, criminality, incarceration, and suicide.[3–5] Thus, childhood behavior problems are a public health challenge in need of significant attention and resources.

Roughly 13 million American children live in poverty,[6] where they are up to three times more likely to develop poor mental health before adulthood.[7,8] However, the pathogenesis of child behavior problems as related to poverty or, more broadly, social disadvantage remains controversial in part because mechanisms underlying how living in low resourced environments leads to behavior problems remain understudied. Understanding why poverty is so strongly linked to increased risk of childhood behavior problems is complicated by poverty's complex ecology of biological, social, and psychosocial risk factors.[9–11]

At all stages of development—beginning before conception, during prenatal and perinatal periods, and through post-natal early life and onward—children growing up in or near poverty are more likely than their more advantaged counterparts to encounter numerous unhealthy exposures, including violence, unstable family environments, high levels of environmental pollution, food insecurity, inadequate housing, and both acute and chronic psychosocial stress from a spectrum of sources. These exposures converge and are embodied in multiple, interacting biological processes, including those involved in neural growth, inflammation, and neuroendocrine stress response.[11] At the same time, these risk factors can co-occur and interact

with protective factors, which have the potential to buffer effects of harmful poverty-related exposures. A variety of potential protective factors have been identified, including those that are intrinsic (e.g., genetic)[12] and extrinsic (e.g., healthy family functioning).[13] These factors may also interact with one another to affect multiple biological domains throughout development, further complicating the study of poverty and health.

To date, a substantial body of research has focused on identifying childhood social environmental risk and protective factors affecting the development of child behavior problems. However, less work has focused on identifying or evaluating mechanisms underlying these associations. The study of mechanisms is important because mechanistic insight sheds light on which social exposures affect health, in what ways, and how, and it also helps direct future research, target resources, and guide the development of public health interventions. Efforts to understand biological mechanisms underlying how social environmental risk and protective factors affect behavioral outcomes have pointed to alterations in brain biology.

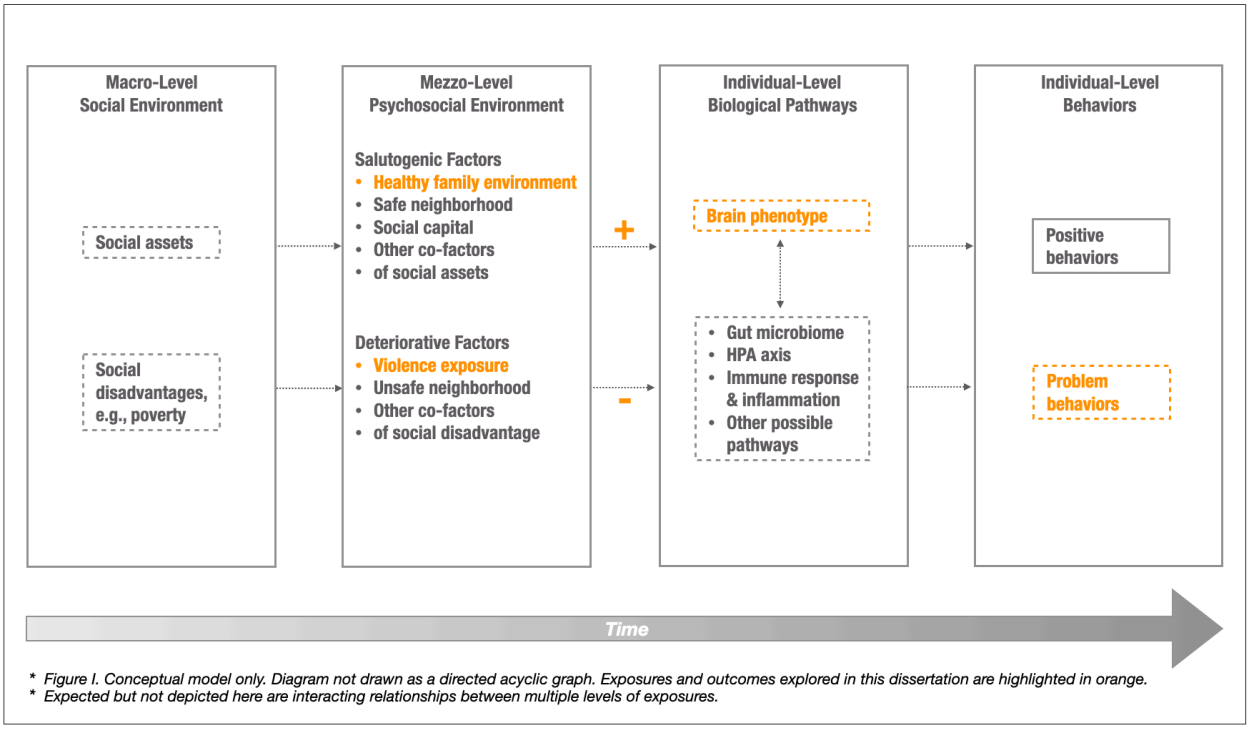
Observational studies have linked negative experiences that commonly occur among individuals with early life social disadvantage to detectable changes in brain morphology and connectivity.[14,15] A comparatively smaller body of research has evaluated potential effects of positive social exposures on brain development, including parental sensitivity and maternal attachment.[16,17] However, neuroimaging research exploring the effects of childhood social exposures has been limited by several factors. Such studies generally rely on small case-control samples using retrospectively reported data, which may lead to various types of selection and reporting bias. Moreover, though research demonstrates the importance of investigating multiple measures of brain structure and function to understand behavior, very few studies have assessed

effects of social environmental risk and protective factors on brain white matter structure with sufficient sample size to draw convincing conclusions.[15,18]

The animating premise of this dissertation is that childhood social advantage and disadvantage are embodied in brain structure and manifest as behavior. This premise is built on aspects of two conceptual frameworks. The first is Bronfenbrenner's Ecological Systems Model, which makes explicit how nested levels of exposures (i.e., individual, family, community, society, etc.) interact to influence child development.[19] The model posits that exposures at each level can either promote or damage health and development. For example, aspects of an unhealthy family environment (e.g., high levels of family conflict) have been associated with poor developmental outcomes, while elements of a healthy family environment (e.g., positive parenting practices) have been associated with positive outcomes beyond those associated with a mere absence of negative family exposures.[20–24] Notably, health-promoting exposures at all levels are under explored. For example, most research on healthy aspects of the family environment focuses on narrowly defined facets related to parenting. Overall family functioning—essentially a composite measure meant to capture positive or negative functioning across multiple domains, including parental support, acceptance, and problem-solving—may more accurately capture the lived experiences of children.

However, because Bronfenbrenner's model does not explicitly consider neurodevelopmental effects of exposures, this dissertation also incorporates aspects of the model of childhood adversity proposed by McLaughlin and Sheridan (2014).[25–27] Their model entails a two-dimensional framework, in which most childhood adverse experiences can be classified as those involving either "threat" (i.e., the *presence* of physically threatening experiences) or "deprivation" (i.e., the *absence* of critical experiential inputs). They further propose that

experiences of threat and deprivation, though often co-occurring, nevertheless exert distinct influences on development, a premise supported by animal models and, increasingly, human studies.[25–28] Their threat-and-deprivation model, however, does not account for interacting exposures at multiple levels, a limitation this dissertation attempts to address by incorporating aspects of the ecological systems model. See Figure I for a schematic diagram of this dissertation's conceptual model.



* *Figure I. Conceptual model only. Diagram not drawn as a directed acyclic graph. Exposures and outcomes explored in this dissertation are highlighted in orange.*
* *Expected but not depicted here are interacting relationships between multiple levels of exposures.*

Research reported in this dissertation leverages methods from population neuroscience and existing neuroimaging data from approximately 3,000 children in the Generation R Study, a population-based birth cohort in Rotterdam, the Netherlands, to measure the brain-based effects of both positive and negative aspects of the social environment. In Chapter 1, we explore potential effects of a positive social environment by considering the effect of healthy family functioning on global measures of brain white matter microstructure. In Chapter 2, we explore potential effects of a negative social environment by considering exposure to various physically

threatening experiences, including exposure to actual violence and mere threatened violence, and its effects on brain morphology. Finally, in Chapter 3, we investigate mediation and moderation of the relationships explored in Chapter 2, including whether and to what extent positive social exposures (e.g., healthy family functioning) buffer the effects of negative exposures (e.g., actual violence exposure) on brain morphology.

Chapter 1

Prenatal family functioning is associated with offspring white matter microstructure in preadolescence.

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Abstract

Background

The origins of child behavior disorders remain poorly understood. Stressful social environments may contribute to their onset, while enriched environments may promote healthy development beyond the mere absence of stressful environments. The family environment is central to the early-life social environment, but research investigating white matter neurodevelopmental pathways potentially explaining associations between the family environment and behavior remains limited. This study tested our hypothesis that healthier early-life family functioning would be associated with higher global fractional anisotropy (FA) and lower global mean diffusivity (MD) in preadolescence, which have previously been associated with fewer behavior problems.

Methods

We analyzed data from 2,653 children in the Generation R Study in Rotterdam, the Netherlands. The study asked mothers to report family functioning using the 12-item McMaster Family Assessment Device, General Functioning at two time points: prenatally (mean gestational age 24 weeks) and in mid-childhood (mean age 6.0 years). Later, the study collected diffusion-weighted scans in preadolescence (mean age 10.1). We computed standardized global FA and MD values by averaging metrics from 27 white matter tracts. We used OLS models to examine both global and tract-specific outcomes adjusting for child sex, age, ethnicity, household income, in utero smoking exposure, and parental education, psychosis history, and psychopathology symptoms.

Results

In fully adjusted, weighted models, a one-unit increase in healthy prenatal family functioning was associated with a 0.13 standard deviation decrease in global MD (95% CI [-0.25, -0.02]). We found no evidence of an association between prenatal functioning and global FA or mid-childhood functioning and either global outcome. Tract-specific analyses supported these global findings.

Conclusion

Healthy early-life family functioning may induce white matter microstructural differences in preadolescence linked previously to reduced problem behaviors.

Introduction

The origins of child behavior disorders remain poorly understood. Increasingly, investigators have called for a population neuroscience approach both to identify factors shaping brain structure and function, and to understand how variations in the brain cause child behavior problems.[29,30] Empirical studies suggest elements of the social environment impact brain development in both positive and negative ways, with effects on aspects of brain function that have been linked with behavior problems.[31–33] Neuroscience research often characterizes the childhood social environment as a monolithic experience measured by childhood socioeconomic status. In contrast, social scientific models of the social environment include experiences related to one's family, friends, schools, organized activities, neighborhood, and place of worship.[19] The relative importance of these domains may change throughout childhood, with the family environment most influential early in life. As such, a healthy early-life family environment may drive healthy brain development and protect against behavior disorders.

However, the neurodevelopmental effects of family-based exposures have not been thoroughly explored. Among studies in this area, most focus on family dysfunction and its link to poor outcomes. For example, a broad spectrum of research links child abuse and maltreatment, which occur most often within the family environment, to structural alterations in corticolimbic regions of the brain involved in cognitive and affective processes underlying behavior problems.[20,34,35] Similarly, functional imaging studies support a positive association between family conflict and adolescent risk-taking behavior.[21,36]

In contrast to research on family dysfunction, some neurodevelopmental studies assess positive family-based experiences, which may confer benefits beyond those linked to a mere absence of negative exposures. For example, greater maternal support and positive parenting

behavior have been associated with brain structural changes thought to be advantageous, including accelerated hippocampal growth in childhood and adolescence, and attenuated amygdala growth in adolescence.[22,37] Some functional imaging studies also report associations between healthy parent-child relationships, decreased risk taking behavior, and increased cognitive control in adolescence and early adulthood.[23,38,39]

These studies are limited insofar as they focus on parenting practices—typically maternal practices—rather than on broader measures of overall family functioning that may capture important characteristics within a complex family ecology. Many of these studies also assess aspects of the family environment during a narrow time period in a child's life. As a result, they cannot quantify how the family environment's influence may change throughout childhood. And despite the importance of white matter to healthy brain development, prior imaging studies of family-based exposures assess only brain functional or structural outcomes.

Studies suggest both negative and positive experiences occurring prenatally, postnatally, and in childhood alter white matter structural development.[40,41] These studies generally report associations between negative exposures (e.g., maternal prenatal anxiety) and properties of white matter microstructure that may decrease neural efficiency, and between positive ones (e.g., breastfeeding) and the opposite.[42–46] Separately, mostly cross-sectional studies report associations between properties of white matter microstructure and behavioral outcomes. In these studies, microstructural properties related to more efficient neural processing are generally associated with fewer behavior problems, while microstructural properties related to less efficient neural processing are associated with antisocial behavior, ADHD, bipolar disorder, and disruptive behavior problems.[41,47,48]

To investigate whether a positive family environment may impact white matter microstructure, this study used prospective data from the Generation R birth cohort, a population-based study tracking child development from pregnancy through adolescence. Study staff collected data on family functioning from mothers prenatally and in mid-childhood, and their children completed an MRI brain scan as preadolescents. We hypothesized that more positive family functioning at each time point would be associated with greater global white matter microstructure, even after extensive adjustment for plausible confounders selected based on prior literature and theory.[41]

Methods

Participants.

This study uses data from the Generation R Study, a prospective, population-based birth cohort in Rotterdam, the Netherlands, seeking to identify social, environmental, and genetic factors affecting child health and development.[49] The Generation R Study enrolled 8,880 pregnant women living in Rotterdam between 2002 and 2006 and another 898 women at the birth of their child during the same time period. Study administrators have collected data through clinic visits and postal questionnaires from children and their caregivers at multiple time points through the present after securing written informed consent and assent from all participants. All study protocols are approved by the Medical Ethics Committee of the Erasmus Medical Center.

Women completed a postal questionnaire about their family functioning prior to the birth of their enrolled child (gestational age range 18 – 25 weeks) and again when their child was in mid-childhood (mean age 6.0 years; range 4.0 – 9.1 years). Mothers enrolled at the birth of their child (and not while pregnant) completed only the mid-childhood questionnaire. In sum, 8,271

women completed at least one of the questionnaires. Later, study researchers obtained diffusion-weighted magnetic resonance imaging (DWI) scans from 3,992 children with mean age 10.1 years (preadolescence; range 8.6 to 12.0 years).[50] The current study included participants if they had a usable DWI scan with no missing tract-specific scalar data (described below) and either prenatal or mid-childhood family functioning data. Among these participants, we excluded those whose mothers reported using cocaine or heroin while pregnant. And because Generation R includes a number of twins and triplets, we randomly selected only one sibling for inclusion in these cases. Our final analysis sample included 2,653 children.

Measures.

Family Functioning

To measure family functioning, parents completed the McMaster Family Assessment Device, General Functioning Subscale.[51] This is a self-report survey of established reliability and validity in Dutch and several other populations, in which parents respond on a 4-point Likert scale to 6 positively-framed and 6 negatively-frame items.[52–54] Representative questions include, "If there are problems, we can count on each other for support," and, "There are a lot of unpleasant and painful feelings in our family." Because these questions do not reference specific family members or roles, parents can respond regardless of their family's structure. We derived a family functioning score at each time point by reverse-scoring negatively framed items, then averaging response scores across all 12 items to yield a family functioning score range of 1 to 4 for each participant and time period, where higher scores indicated more positive functioning. Cronbach's alpha in the analytic sample was strong (0.89) at both prenatal and mid-childhood time periods.

Brain Imaging

Generation R researchers have described diffusion-weighted imaging collection protocols and preprocessing pipelines elsewhere.[50] All scans were acquired by a GE MR-750W scanner (General Electric Healthcare, Chicago, IL) at 3T with an eight-channel head coil. Sequence parameters yielded 2 mm isotropic resolution and 35 diffusion-weighted volumes. Study staff preprocessed the resulting images using the FMRIB Software Library (FSL), version 5.0.9, to calculate voxel-specific scalar metrics of white matter microstructure, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). Next, they used the FSL AutoPtx plugin to compute tract-specific scalar metrics for 27 large white matter tracts, including three brainstem tracts (middle cerebellar peduncle; left and right medial lemniscus), ten projection fibers (left and right corticospinal tracts and acoustic radiations, and bilateral anterior, posterior, and superior thalamic radiations), eight association fibers (bilateral superior and inferior longitudinal fasciculi, and bilateral inferior fronto-occipital and uncinate fasciculi), four limbic system fibers (left and right cingulate gyrus part of the cingulum and parahippocampal part of the cingulum), and two callosal fibers (forceps major and forceps minor).[55] See Supplement Section 1.1 for more detail information about scan acquisition and processing. To ensure the quality of all scans and reconstructions, researchers visually inspected all raw images and examined signal intensity in each slice to assess attenuation by various artifacts. They also visually inspected probabilistic tractography data. Scans deemed poor at any point in the quality control process were excluded from analysis.

Following prior research on white matter microstructure, we focused our primary analyses on two measures, FA and MD.[56,57] FA assesses the extent to which white matter microstructure constrains water molecule diffusion in a single direction. MD is a measure of the

extent to which water molecules in white matter move freely in all directions. In secondary analyses, we also assessed AD and RD, which measure how much water molecules are able to move in specific directions. All four measures provide complementary information from which inferences about white matter microstructural anatomy can be made. As children age, FA values increase, and MD values decrease.[41] Higher FA and lower MD values suggest more organized white matter, which in turn may enable more efficient neural functioning.[41]

Because complex human cognition manifests from coordinated activity across many functionally distinct brain regions, we constructed "global" measures of white matter microstructure incorporating information from all 27 tracts delineated by AutoPtx. Specifically, we constructed global scalar metrics by calculating the mean of the weighted-average FA, MD, AD, and RD values, then standardizing the resulting means. Separately, for the 24 tracts with analogues in both hemispheres (e.g., left and right uncinate fasciculus), we averaged and standardized measures (FA, MD, AD, and RD) from both hemispheres. For example, we averaged left and right FA values for each participant's uncinate fasciculi, resulting in a single mean FA value for the uncinate fasciculus. Because three tracts (middle cerebellar peduncle, forceps major and minor) do not have independent analogues in both hemispheres, this process resulted in 15 sets of tract-specific values used in our analyses.

Covariates

Researchers retrieved child birthdate and sex data from birth records. Parents self-reported the following: their country of origin and ethnicity, which we used to categorize child ethnicity as non-Turkish European (including Dutch), Turkish, Moroccan, and Other Ethnicity; household income during pregnancy (more or less than €2200 / month); highest maternal and 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); maternal age at childbirth; maternal smoking history during pregnancy (never, until pregnancy was known, or through pregnancy); and parental psychopathology symptoms prenatally (for models of prenatal family functioning; measured using the full 53-item Brief Symptom Inventory (BSI)) and at child-age 3 years (for models of mid-childhood family functioning; measured using a subset of 21 items from the BSI).[58] We calculated continuous BSI sum scores for each parent at each time point.

Statistical Analyses.

We assessed and removed as appropriate outliers in FA, MD, AD, and RD using standard methods (n = 161 removed; see Supplement Section 1.2).

To investigate whether family functioning was associated with our primary measures of white matter microstructure (i.e., FA and MD only), we used ordinary least squares linear regression. We imposed a hierarchical structure to these analyses with initial models examining global outcomes and subsequent models evaluating specific tracts, for which we adjusted p-values for multiple comparisons via the Bonferroni method. For each outcome, we fit (1) unadjusted models; (2) minimally adjusted models accounting for each child's age at DWI scan, sex at birth, and ethnicity; and (3) fully adjusted models incorporating all other covariates listed above. We ran separate models to assess associations with prenatal and mid-childhood family functioning. In secondary analyses, we considered models that included measures of family functioning at both time points simultaneously. Finally, we fit fully adjusted models weighted to account for differential attrition (see below for details).

We conducted several sensitivity analyses. First, we evaluated whether prenatal family functioning modified effects of mid-childhood family functioning by incorporating an interaction term between prenatal and mid-childhood functioning scores using continuous measures in fully adjusted models. Second, we calculated a mean family functioning score over time (i.e., the mean score of both time points) and evaluated associations between mean family functioning and global outcomes. Third, because there was substantial left skew in the functioning score distributions (see below for more detail), we fit fully adjusted piecewise continuous linear spline models of prenatal functioning and both global outcomes. Based on *a priori* considerations of the family functioning scale and score distributions in our sample, we initially modeled a knot at a score of 3.0, after which we iteratively modeled alternative knots below 3.0 in functioning score decrements of 0.1.

Missing data.

To account for differential loss to follow-up by important sociodemographic characteristics, we calculated inverse probability of attrition weights (IPWs). For purposes of this study, participants enrolled at baseline but excluded from our analysis sample for any reason were deemed lost to follow-up. We multiply imputed missing exposure and covariate data using chained equations to construct 50 imputed datasets, then combined imputation-specific mean and variance measures for each imputed variable using Rubin's Rules.[59] See Supplement Sections 1.3 and 1.4 for additional details of our IPWs and imputation models.

Results

Analytic sample characteristics.

Included versus excluded participants were more likely to be of non-Turkish European ethnicity (71% vs. 58%); to have parents with at least advanced vocational training or a bachelor's degree (63% vs. 44%); to be from higher-income households (65% vs. 49%); and to be born to older mothers (mean maternal age at birth 31.7 years vs. 29.8 years).

Table 1.1 details sociodemographic characteristics in our analytic sample according to family functioning scores. Mothers of Dutch / other European children reported higher family functioning scores at both time points than mothers of children of other ethnicities, as did mothers of higher-income households and households of higher education. Prenatal and mid-childhood scores were moderately correlated, $r = 0.38$. Functioning scores at both time points were left skewed. The prenatal mean and median scores were 3.48 and 3.58, respectively, with 75% of mothers in the analysis sample reporting scores greater than or equal to 3.0. Similarly, the mid-childhood mean and median scores were 3.50 and 3.58, respectively, while fully 83% of mothers reported mid-childhood scores 3.0 or higher.

Table 1.1. Distribution of exposure measures by participant characteristics in the final analysis sample. n = 2,653.

		Prenatal Family Functioning		Mid-Childhood Family Functioning	
	%	$\bar{x}$	*s*	$\bar{x}$	*s*
Total Sample	100	3.48	0.46	3.50	0.42
Child biological sex					
Female	51	3.49	0.46	3.51	0.41
Male	49	3.47	0.46	3.49	0.43
Child race / ethnicity / country of origin					
Dutch / Other European	71	3.56	0.42	3.55	0.40
Turkish	5	3.26	0.48	3.29	0.49
Moroccan	4	3.26	0.47	3.27	0.40
Surinamese	7	3.25	0.50	3.39	0.45
Other	13	3.30	0.53	3.40	0.43
Highest Household Education					
Less than high school equivalent	4	3.18	0.49	3.22	0.47
High school or intermediate vocational trainin	33	3.36	0.49	3.44	0.43
Adv. vocational training, bachelor's, or higher	63	3.60	0.41	3.56	0.40
Household Income					
€2200 / month or less	35	3.30	0.51	3.38	0.46
More than €2200 / month	65	3.59	0.40	3.57	0.38

1. This table is based on observed values for each characteristic and does not account for missing data.
2. Family functioning scores are based on the McMaster Family Assessment Device - General Functioning Subscale and range from 1 to 4.

On average, girls had lower global FA and MD scores than boys (p-values < 0.001 for both outcomes). Dutch / other European children had higher global FA values than children of other ethnicities ($p < 0.001$). Children of more socially advantaged households had higher global FA values than their less advantaged counterparts ($p = 0.002$ and < 0.001 for parental education and household income, respectively). No differences in global MD by ethnicity, parental education, or household income were evident.

Global outcomes.

In an unweighted, fully adjusted model, prenatal family functioning was negatively associated with preadolescent global MD (Table 1.2), with modest evidence of a positive association with global FA. The magnitudes of the prenatal functioning effect estimates were approximately 57% and 36% of those associated with a one-year increase in age at scan for global MD and global FA, respectively. Weighted models revealed similar results, though standard errors for both outcomes were greater than in unweighted models. In contrast, we found no evidence for an association between mid-childhood functioning and either measure of white matter microstructure. Notably, in models of mid-childhood functioning that adjusted for prenatal functioning, prenatal functioning remained a significant predictor of both outcomes. For example, in unweighted, fully adjusted models of mid-childhood functioning, effect estimates for prenatal functioning were $\beta_{\text{global FA}} = 0.12$ (95% CI: 0.02, 0.22) and $\beta_{\text{global MD}} = -0.13$ (95% CI: -0.23, -0.03). See Supplement Section 1.5 for results of models of global AD and RD.

Table 1.2. Associations between family functioning and global measures of white matter microstructure in preadolescence. n = 2,653.

		Global FA			Global MD		
		β	95% CI	*p*	β	95% CI	*p*
Prenatal Family Functioning							
Unadjusted	Unweighted	0.17	(0.08, 0.26)	< 0.001	-0.10	(-0.19, -0.01)	0.02
Fully adjusted	Unweighted	0.09	(-0.01, 0.19)	0.07	-0.13	(-0.23, -0.04)	0.01
Fully adjusted	Weighted	0.09	(-0.03, 0.20)	0.13	-0.13	(-0.25, -0.02)	0.03
Mid-Childhood Family Functioning - Baseline Unadjusted							
Unadjusted	Unweighted	0.04	(-0.05, 0.13)	0.44	-0.02	(-0.12, 0.07)	0.65
Fully adjusted	Unweighted	-0.02	(-0.12, 0.08)	0.68	-0.01	(-0.11, 0.09)	0.85
Fully adjusted	Weighted	-0.05	(-0.17, 0.07)	0.42	0.00	(-0.12, 0.12)	0.96
Mid-Childhood Family Functioning - Baseline Adjusted							
Unadjusted	Unweighted	-0.04	(-0.14, 0.06)	0.45	0.02	(-0.08, 0.13)	0.66
Fully adjusted	Unweighted	-0.06	(-0.16, 0.05)	0.27	0.03	(-0.07, 0.13)	0.55
Fully adjusted	Weighted	-0.09	(-0.21, 0.04)	0.17	0.04	(-0.08, 0.16)	0.54

1. Unadjusted models include no covariates unless specifically noted.
2. Fully adjusted models account for child age at scan, biological sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, prenatal maternal and partner psychopathology symptoms (for prenatal models), early-childhood maternal and partner psychopathology symptoms (for mid-childhood models), maternal age at child's birth, and child in utero exposure to smoking.
3. Global measures are standardized mean values of weighted-average FA and MD across all 27 tracts delineated by AutoPtx.

Tract-specific outcomes.

Exploratory tract-specific models revealed associations between prenatal functioning and MD in the uncinate fasciculus, medial lemniscus, parahippocampal part of the cingulum, and forceps major; however, the latter two associations did not survive Bonferroni correction for multiple testing (Table 1.3). The remaining tract-specific MD effect estimates had larger standard errors and thus did not evince associations as measured strictly by statistical significance, but all MD effect estimates were uniform in direction (Supplement Figure 1.1). A similar pattern emerged from models assessing prenatal functioning and tract-specific FA: effect estimates were nearly uniform in directionality, though only the association with medial lemniscus FA remained statistically significant after Bonferroni correction (Table 1.3,

Supplement Figure 1.2). For post-hoc exploration of tract-specific models assessing effects of mid-childhood functioning, see Supplement Section 1.7.

Table 1.3. Unweighted associations between prenatal family functioning and tract-specific measures of white matter microstructure.

	Fractional Anisotropy			Mean Diffusivity		
	β	95% CI	*p*	β	95% CI	*p*
Association Fibers						
Superior Longitudinal Fasciculus	0.09	(-0.00, 0.19)	0.06	-0.08	(-0.17, 0.02)	0.10
Inferior Longitudinal Fasciculus	0.06	(-0.04, 0.15)	0.26	-0.08	(-0.17, 0.02)	0.11
Inferior Fronto-Occipital Fasciculus	0.00	(-0.10, 0.10)	0.98	-0.06	(-0.16, 0.03)	0.20
Uncinate Fasciculus	0.01	(-0.09, 0.11)	0.85	-0.19	(-0.29, -0.10)	< 0.001
Limbic System Fibers						
Cingulum (Cingulate Gyrus Part)	0.04	(-0.05, 0.14)	0.38	-0.09	(-0.19, 0.01)	0.07
Cingulum (Parahippocampal Part)	0.07	(-0.03, 0.16)	0.16	-0.11	(-0.21, -0.01)	0.03
Projection Fibers						
Corticospinal Tract	0.08	(-0.02, 0.17)	0.12	-0.06	(-0.16, 0.04)	0.22
Acoustic Radiation	0.03	(-0.07, 0.13)	0.52	-0.05	(-0.15, 0.05)	0.33
Anterior Thalamic Radiation	0.05	(-0.05, 0.14)	0.34	-0.08	(-0.18, 0.01)	0.09
Superior Thalamic Radiation	0.04	(-0.06, 0.13)	0.48	-0.07	(-0.16, 0.03)	0.17
Posterior Thalamic Radiation	-0.02	(-0.12, 0.08)	0.68	-0.01	(-0.11, 0.09)	0.82
Callosal Fibers						
Forceps Major	0.07	(-0.03, 0.17)	0.16	-0.10	(-0.20, -0.01)	0.04
Forceps Minor	0.04	(-0.06, 0.14)	0.43	-0.07	(-0.16, 0.03)	0.19
Brainstem Tracts						
Middle Cerebellar Peduncle	0.08	(-0.02, 0.17)	0.13	-0.03	(-0.12, 0.07)	0.60
Medial Lemniscus	0.18	(0.09, 0.27)	< 0.001	-0.19	(-0.28, -0.09)	< 0.001

1. Fully adjusted models account for child age at scan, biological sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, maternal and partner psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.
2. FA and MD measures are standardized.

Sensitivity analyses.

In fully-adjusted models of mid-childhood functioning in relation to FA and MD, we found no evidence of statistical interaction between prenatal and mid-childhood functioning scores (interaction terms: $\beta_{\text{global FA}} = 0.13$, 95% CI, -0.09, 0.35; $\beta_{\text{global MD}} = -0.14$, 95% CI, -0.36,

0.09). In models assessing mean family functioning with global outcomes, mean family functioning was not associated with either outcome ($\beta_{\text{global FA}}$ = 0.04, 95% CI, -0.08, 0.16; $\beta_{\text{global MD}}$ = -0.10, 95% CI, -0.22, 0.02). Piecewise continuous linear spline models suggested effects of greater magnitudes for higher prenatal functioning scores, i.e., between 3.0 and 4.0, where most of our participants were clustered. For the relatively fewer number of participants with lower functioning scores (i.e., between 1.0 and 3.0), spline model effect estimates were more uncertain. See Supplement Section 1.8 for more details.

Discussion

This study provides evidence to support our hypothesis that early-life family functioning may affect white matter neurodevelopment. Specifically, more positive prenatal family environments (i.e., supportive and accepting families with high problem-solving capacity) were associated with lower MD values, on average, across the brain in preadolescence. While the magnitudes of the effect estimates were relatively small in absolute terms, they can be compared to other known contributors to white matter microstructure. For example, the difference in global MD associated with a one-unit increase in prenatal family functioning score was roughly half that associated with a one-year increase in child age at scan. The three-unit range of the family functioning scale (i.e., from 1 to 4) renders these estimates more substantial when comparing children of families with exceedingly low scores to those with very high scores. In addition, certain tract-specific effect estimates were larger. In the uncinate fasciculus, a one-unit increase in prenatal functioning score was associated with a larger decrease in MD than a one-year increase in scan age. In contrast to our findings with prenatal family functioning, we found no

evidence suggesting a relationship between mid-childhood family functioning and our global outcomes.

Secondary analyses suggest effects of prenatal family functioning on mean diffusivity may be widespread throughout the brain. Though only MD estimates of the uncinate fasciculus and medial lemniscus among the 15 tract-specific outcomes tested survived Bonferroni adjustment, the uniform directionality and similar magnitude of the remaining tracts' estimates suggest a model of global effects rather than one in which effects are targeted at specific tracts. Moreover, if effects were targeted at specific tracts, one might postulate the uncinate fasciculus and medial lemniscus would share a common structural feature or functional role. Yet, this does not appear to be true. The uncinate fasciculus connects the temporal and frontal lobes in the brain and is involved in memory, language, and social-emotional processing.[60] The medial lemniscus is a brainstem tract involved in sensory information transport to the brain.[61] And while both tracts appear to emerge around the same time at 15 gestational weeks, many other tracts for which effect estimates were not strictly statistically significant also appear to emerge between 13 and 19 gestational weeks.[62] Thus, considered in their totality, our tract-specific analyses suggest prenatal family functioning may have global rather than targeted effects.

Our findings are consistent with the limited available prior work in this area. The only other study to assess prenatal and early childhood life experiences and white matter microstructure in a population-based cohort also found lasting effects of prenatal exposures. Using DWI scans obtained when participants were in early adulthood, Jensen et al. (2018) reported maternal prenatal stressful experiences were associated with a decrease in the magnetization transfer ratio (MTR) in the splenium. Notably, as MTR decreases, MD generally increases. Moreover, our results support findings from prior studies reporting positive parenting

practices or healthy parent-child relationships confer neurodevelopmental advantages associated with decreased risky behavior. Because many of these studies assess the family environment after the children are born, they are vulnerable to reverse causation, since child behavior likely influences family functioning. Our study, however, found similar effects using a measure of prenatal family functioning obtained before the child's birth, thereby reducing concerns about recall bias and reverse causation. Together, these findings suggest additional investigation is warranted to explore whether, how, and to what extent early-life experiences may impact white matter development over time.

The period from the last weeks of gestation through the first years of life is critical to a number of foundational white matter developmental processes, which may be affected by the family environment and may also explain lasting microstructural differences. Our prenatal measure of the family environment is unlikely to measure the prenatal environment exclusively. Rather, it more likely captures the perinatal and early-childhood family environment, spanning some time period both before and after the child's birth. Interestingly, we found prenatal and mid-childhood functioning scores were only moderately correlated ($r = 0.38$), suggesting that the family environment may change modestly through the child's first six years of life. Follow-up research may investigate whether and to what extent family functioning fluctuates during this time period by recording repeated measures of functioning over shorter intervals. Measures of prenatal and immediate postnatal functioning may be of particular interest as families adjust to the presence of a new infant while the infant continues rapid white matter development.

Jensen et al. (2018) propose at least three complementary mechanisms that may link prenatal stress and white matter microstructure, which we adapt here. The first is the balance between neurogenesis (neuron production) and apoptosis (neuron death). Both processes occur in

the prenatal and, at least within the hippocampus, the very early postnatal period. The balance between these processes affects neuronal density by influencing the number of neurons (and thus axons) that comprise the brain's white matter. Studies in humans and other animals suggest both processes are in part experience dependent. Maternal stress, for example, may reduce neuronal density by decreasing neurogenesis and increasing apoptosis, while enriched environments may increase neuronal density by doing the opposite.[41,42] Increased neuronal density could result in higher FA and lower MD values.[42]

Another possible mechanism is altered developmental myelination, or the process by which axons develop an insulating myelin sheath to enhance their efficiency. Myelination begins in the late prenatal period and extends well into childhood. Enriched environments have been associated with increased FA and decreased MD, which suggest greater myelination. Likewise, stressful environments have been associated with decreased FA and increased MD, which suggest lesser myelination.[42] Positive family functioning may have effects similar to those of enriched environments, in turn producing results similar to those reported here.

A third potential mechanism relates to changes in axonal diameter and the thickness of the myelin sheath. Larger axons have thinner myelin sheaths compared to smaller axons, resulting in different microstructural profiles. Because enriched environments entail novel and healthy stimuli, they may increase neuronal activity and promote axonal growth.[42] Both FA and MD may be influenced by these changes, such that a greater density of large-diameter axons (perhaps resulting from enriched environments) would manifest as higher FA and lower MD.

Our study has limitations. First, the sample included few families reporting low functioning scores, perhaps due either to selection or social desirability bias. This inhibits our ability to examine effects of scores at the low end of the continuum. Second, with only one MRI

scan per participant, we cannot fully assess changes in neurodevelopmental trajectories due to our exposure. Third, as with all observational studies, confounding and reverse causation may bias our results. For example, certain parental genetic profiles may predispose parents to report higher or lower family functioning while also affecting the white matter development of their children. We partly addressed this concern by adjusting for both maternal and paternal psychopathology symptoms and history of psychosis. Fourth, our study is limited by challenges inherent in large, population-based pediatric neuroimaging studies. For example, we excluded a substantial number of participants due to poor scan quality, which can be patterned by child behavior and sociodemographic profiles. Relatedly, the study's generalizability is limited by differential attrition in the cohort by important sociodemographic characteristics, although our use of inverse probability weights to account for attrition helps to address this concern. Finally, because processing pipelines are primarily optimized for adults, rapidly changing myelin densities in preadolescence may have made image reconstruction more difficult, which may increase measurement error in our primary outcomes.

Our study also has several strengths. First, we used a longitudinal design, leveraging prospectively collected exposure data predating the child's birth and linking it to outcomes measured fully ten years after the initial exposure assessment. This substantially mitigates concerns about reverse causation and recall bias. We also avoid many challenges associated with studies using maternal reports of both exposures and outcomes (e.g., cognitive and behavior measures) by using objective outcomes constructed from MRI scans. Separately, by measuring outcomes in preadolescent children, this study is able to investigate relatively long-term effects of the perinatal family environment. Finally, this study is nested within a large, population-based

birth cohort, which reduces the risk of selection bias common to many neuroimaging studies relying on case-control designs.

Conclusion

In a sample of 2,653 children, higher levels of prenatal family functioning—a measure of the general perinatal family environment—were associated with greater white matter microstructure in preadolescent children, suggesting healthy perinatal family functioning may confer neurodevelopmental advantages throughout childhood. Our results also suggest the emphasis on parenting practices in research assessing the impact of family-based exposures may be too narrow, and that more general measures of family functioning agnostic to family structure may capture an important dimension of the family environment. Subsequent studies of family functioning may consider developing new assessment tools to capture variation at both the lower and the higher end of the scale. Future research should also emphasize participation of low-functioning families, leverage repeated MRI scans beginning earlier in childhood when feasible, and focus on white matter microstructure in addition to other markers of brain structure and function.

Appendix of Supplemental Information

Section 1.1. Brain imaging details

Generation R researchers have described diffusion-weighted imaging collection protocols and preprocessing pipelines elsewhere.[50] All scans were acquired by a GE MR-750W scanner (General Electric Healthcare, Chicago, IL) at 3T with an eight-channel head coil. Sequence parameters included 2 mm isotropic resolution and 35 diffusion-weighted volumes. Study staff preprocessed the resulting images using the FMRIB Software Library (FSL), version 5.0.9, which stripped non-brain tissue, corrected for artifacts from eddy currents and head motion, and fit a diffusion tensor to each voxel using the RESTORE method from the Camino diffusion MRI toolkit. This pipeline calculated fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) metrics for each voxel.

Next, study researchers conducted fully automated probabilistic fiber tractography on each participant's diffusion-weighted image in native space using the AutoPtx plugin for FSL.[55] This method generates subject-specific, probabilistic representations of 27 large white matter tracts that can be consistently and robustly identified across brain regions. The process identifies each tract's connectivity distribution, normalizes it given the number of successful seed-to-target attempts, and then removes voxels unlikely to be part of the tract's distribution. The process delineates the following tracts in each hemisphere: three brainstem tracts (middle cerebellar peduncle; left and right medial lemniscus), ten projection fibers (left and right corticospinal tracts and acoustic radiations, and bilateral anterior, posterior, and superior thalamic radiations), eight association fibers (bilateral superior and inferior longitudinal fasciculi, and bilateral inferior fronto-occipital and uncinate fasciculi), four limbic system fibers (left and right cingulate gyrus

part of the cingulum and parahippocampal part of the cingulum), and two callosal fibers (forceps major and forceps minor). Thereafter, the process automatically computes tract-specific scalar metrics of microstructural properties by weighting voxel-specific metrics by the probability that each voxel is part of the specific tract. To ensure the quality of all scans and reconstructions, researchers visually inspected all raw images and examined signal intensity in each slice to assess attenuation by various artifacts. They also visually inspected all probabilistic tractography data. Scans deemed poor at any point in the quality control process were excluded from analysis.

Following prior research on white matter microstructure, we focused on two measures, FA and MD.[56,57] FA assesses the extent to which white matter microstructure constrains water molecule diffusion in a single direction. MD is a measure of the extent to which water molecules in white matter move freely in all directions. Both measures provide complementary information from which inferences about white matter microstructural anatomy can be made. Higher FA and lower MD values suggest more organized white matter, which in turn may enable more efficient neural functioning.[41]

Section 1.2. Outlier analyses.

We assessed statistical outliers in four measures of white matter microstructure: tract-specific FA, MD, AD, and RD. Though our primary outcomes are composite FA and MD metrics, we included AD and RD outcomes in the outlier analyses because they are based more directly on tensor eigenvalues describing diffusion anisotropy than MD and FA and therefore are less likely to obscure extreme values. For example, while MD is the mean of all three tensor eigenvalues (λ_1, λ_2, and λ_3), RD is merely the mean of two (λ_2 and λ_3), and AD is simply λ_1, where $\lambda_1 > \lambda_2 > \lambda_3$.

In our first outlier identification strategy, we excluded participants with any tract-specific FA, MD, AD, or RD value greater than 5 standard deviations from the mean value for each respective tract because such values are either (1) biologically implausible or (2) so far apart from sample means that they do not represent our population of interest (and indeed are likely the result of pathology). Next, we calculated jackknife residuals of minimally adjusted models for the association between prenatal and mid-childhood family functioning and tract-specific FA, MD, AD, and RD outcomes. Using Tukey's formula, we then excluded participants with any jackknife residual beyond Tukey's outer fences, i.e., greater than a cutoff value at 3 interquartile ranges above the respective residual distribution's 75^{th} percentile or below its 25^{th} percentile. When this test identified statistical outliers, we re-ran the original models excluding the outliers and repeated diagnostic testing until the process revealed no additional outlier values. Finally, we visually inspected quantile-quantile plots of all outcomes and excluded any remaining participants with outlier outcome metrics.

Section 1.3. Inverse probability of attrition weights.

We defined participants lost to follow up as those enrolled at baseline (either prior to or at birth) 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 multiple imputation by chained equations (predictive mean matching for all variables, knn = 10, burn-in = 25) to address missing data in these variables, resulting in 50 imputed datasets. Thereafter, we fit logistic regression models using these variables to predict the likelihood of each enrolled participant's inclusion in our analysis sample. Then, we calculated IPWs for use in later analyses.

Section 1.4. Multiple imputation models.

We imputed missing exposure and covariate data. The proportion of missing data for most covariates was low to moderate (e.g., 12% for paternal age at birth), with the exception of household income, for which we were missing 20% of data. We used the 'mi impute chained' package in Stata 16.0/MP. For all variables, we specified predictive mean matching models, knn = 10 (i.e., 10 donor values), and burn-in = 25 iterations for each chain to ensure convergence to a stationary distribution. Models included all outcomes as right-hand side variables with no missing data. We imputed 50 imputed datasets and combined the resulting estimates using Rubin's Rules.[59]

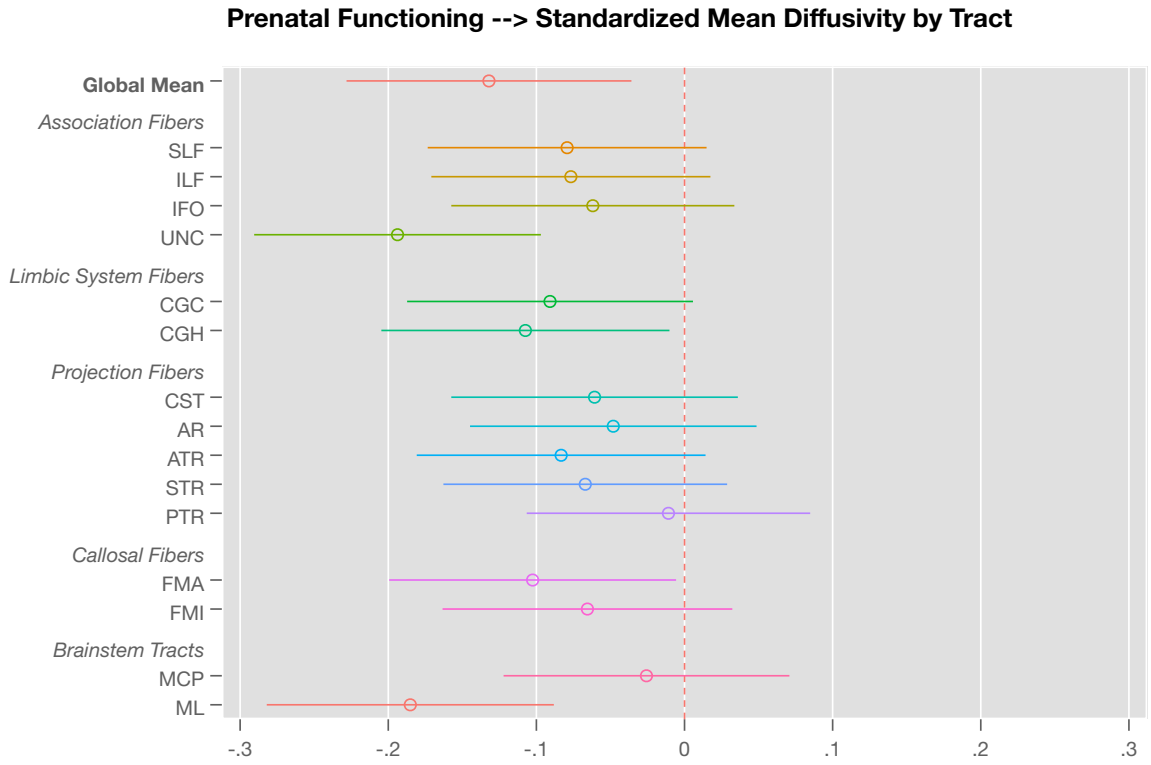
Section 1.5. Global axial and radial diffusivity results.

Supplement Section 1.5. Associations between family functioning and global measures (AD and RD) of white matter microstructure in preadolescence.

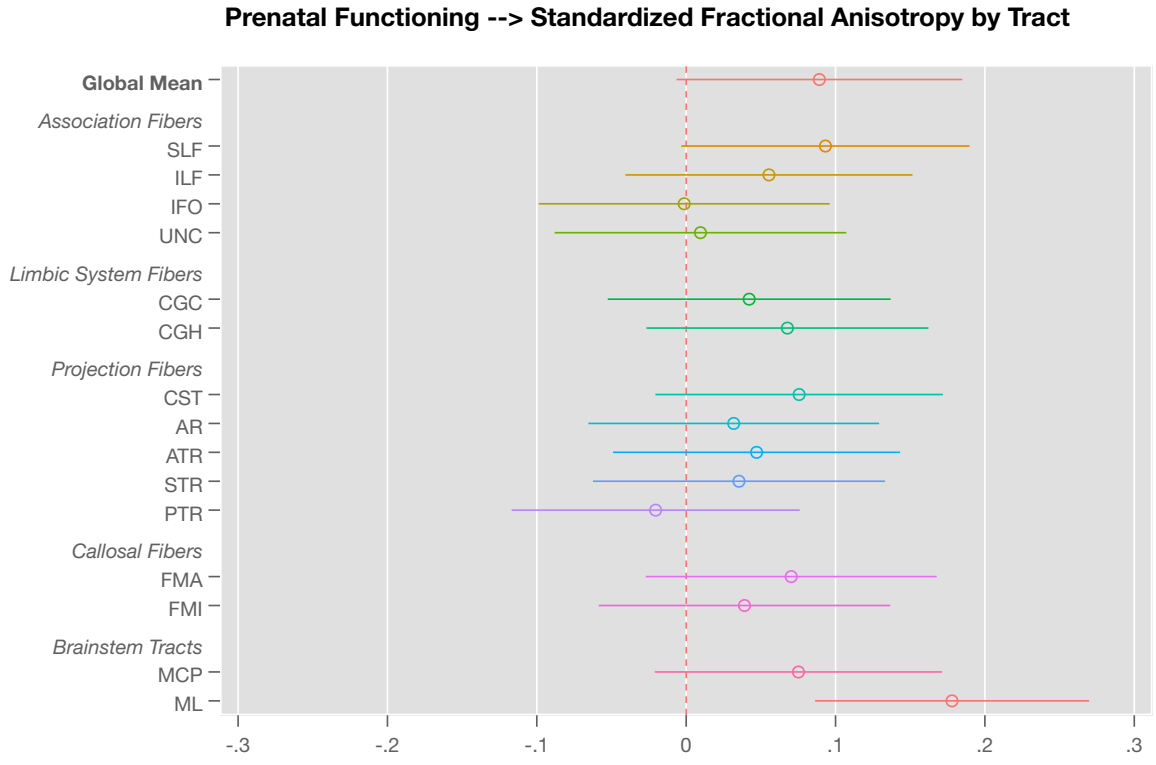
		Axial Diffusivity			Radial Diffusivity		
		β	95% CI	*p*	β	95% CI	*p*
Prenatal Family Functioning							
Unadjusted	Unweighted	0.02	(-0.06, 0.11)	0.59	-0.15	(-0.24, -0.07)	0.00
Fully adjusted	Unweighted	-0.08	(-0.18, 0.02)	0.10	-0.13	(-0.23, -0.04)	0.01
Fully adjusted	Weighted	-0.08	(-0.20, 0.05)	0.21	-0.14	(-0.25, -0.02)	0.02
Mid-Childhood Family Functioning - Baseline Unadjusted							
Unadjusted	Unweighted	0.01	(-0.09, 0.10)	0.86	-0.04	(-0.13, 0.06)	0.46
Fully adjusted	Unweighted	-0.03	(-0.12, 0.07)	0.59	0.00	(-0.10, 0.10)	0.96
Fully adjusted	Weighted	-0.04	(-0.16, 0.09)	0.57	0.03	(-0.09, 0.14)	0.67
Mid-Childhood Family Functioning - Baseline Adjusted							
Unadjusted	Unweighted	0.00	(-0.10, 0.10)	0.97	0.03	(-0.07, 0.14)	0.52
Fully adjusted	Unweighted	-0.01	(-0.11, 0.10)	0.86	0.05	(-0.06, 0.15)	0.36
Fully adjusted	Weighted	-0.03	(-0.16, 0.11)	0.71	0.07	(-0.05, 0.19)	0.26

1. Unadjusted models include no covariates unless specifically noted.
2. Fully adjusted models account for child age at scan, biological sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, prenatal maternal and partner psychopathology symptoms (for prenatal models), early-childhood maternal and partner psychopathology symptoms (for mid-childhood models), maternal age at child's birth, and child in utero exposure to smoking.
3. Global measures are mean values of weighted-average FA and MD across all 27 tracts delineated by AutoPtx.

Section 1.6.1. Supplement figure 1.1



Section 1.6.2. Supplement figure 1.2



Section 1.7. Tract-specific results of mid-childhood family functioning.

Supplement Section 1.7. Unweighted associations between mid-childhood family functioning and tract-specific measures of white matter microstructure.

	Fractional Anisotropy			Mean Diffusivity		
	β	95% CI	*p*	β	95% CI	*p*
Association Fibers						
Superior Longitudinal Fasciculus	-0.07	(-0.18, 0.03)	0.16	0.07	(-0.03, 0.17)	0.17
Inferior Longitudinal Fasciculus	-0.09	(-0.12, 0.01)	0.08	0.04	(-0.06, 0.14)	0.48
Inferior Fronto-Occipital Fasciculus	-0.08	(-0.18, 0.03)	0.14	0.02	(-0.08, 0.13)	0.66
Uncinate Fasciculus	-0.09	(-0.20, 0.01)	0.09	0.02	(-0.08, 0.13)	0.68
Limbic System Fibers						
Cingulum (Cingulate Gyrus Part)	-0.01	(-0.11, 0.10)	0.91	0.07	(-0.03, 0.18)	0.16
Cingulum (Parahippocampal Part)	-0.06	(-0.16, 0.04)	0.24	0.03	(-0.08, 0.13)	0.62
Projection Fibers						
Corticospinal Tract	0.01	(-0.09, 0.12)	0.81	-0.01	(-0.12, 0.09)	0.79
Acoustic Radiation	-0.10	(-0.20, 0.01)	0.07	0.01	(-0.10, 0.11)	0.88
Anterior Thalamic Radiation	-0.01	(-0.11, 0.10)	0.91	0.01	(-0.10, 0.11)	0.91
Superior Thalamic Radiation	0.04	(-0.07, 0.14)	0.46	0.01	(-0.09, 0.12)	0.79
Posterior Thalamic Radiation	-0.07	(-0.17, 0.04)	0.22	0.02	(-0.09, 0.12)	0.76
Callosal Fibers						
Forceps Major	-0.09	(-0.19, 0.02)	0.10	0.06	(-0.05, 0.16)	0.27
Forceps Minor	0.03	(-0.07, 0.14)	0.52	-0.10	(-0.20, 0.01)	0.06
Brainstem Tracts						
Middle Cerebellar Peduncle	-0.02	(-0.12, 0.09)	0.77	-0.04	(-0.14, 0.07)	0.47
Medial Lemniscus	0.08	(-0.01, 0.18)	0.09	0.05	(-0.06, 0.15)	0.38

1. Fully adjusted models account for prenatal family functioning, child age at scan, biological sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, maternal and partner psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.
2. FA and MD measures are standardized.

Section 1.8. Spline models of prenatal family functioning and primary global outcomes.

Supplement Section 1.8.1. Piecewise continuous linear spline model results, prenatal family functioning and global mean diffusivity.

	Pre-knot		Post-knot	
Knot	β	95% CI	β	95% CI
2.5	-0.05	(-0.59, 0.48)	-0.13	(-0.23, -0.02)
2.6	-0.03	(-0.50, 0.44)	-0.13	(-0.24, -0.02)
2.7	-0.03	(-0.44, 0.38)	-0.13	(-0.25, -0.02)
2.8	-0.07	(-0.42, 0.29)	-0.13	(-0.25, -0.01)
2.9	-0.08	(-0.39, 0.23)	-0.13	(-0.25, -0.01)
3.0	-0.08	(-0.35, 0.20)	-0.13	(-0.26, -0.00)
No knot			-0.13	(-0.23, -0.04)

1. Fully adjusted models account for child age at scan, biological sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, maternal and partner psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.
2. The post-knot β is the estimated absolute slope, i.e., not merely the change in slope relative to the pre-knot β.

Supplement Section 1.8.2. Piecewise continuous linear spline model results, prenatal family functioning and global fractional anisotropy.

	Pre-knot		Post-knot	
Knot	β	95% CI	β	95% CI
No knot			0.09	(-0.01, 0.19)
3.0	0.01	(-0.26, 0.28)	0.12	(-0.01, 0.25)
2.9	-0.04	(-0.34, 0.27)	0.13	(0.00, 0.25)
2.8	-0.04	(-0.39, 0.31)	0.12	(0.00, 0.24)
2.7	-0.03	(-0.43, 0.37)	0.11	(-0.00, 0.23)
2.6	0.00	(-0.46, 0.45)	0.10	(-0.01, 0.21)
2.5	0.03	(-0.49, 0.54)	0.10	(-0.01, 0.21)

1. Fully adjusted models account for child age at scan, biological sex, ethnicity, household income, highest level of parental education achieved, maternal and partner history of psychosis, maternal and partner psychopathology symptoms, maternal age at child's birth, and child in utero exposure to smoking.
2. The post-knot β is the estimated absolute slope, i.e., not merely the change in slope relative to the pre-knot β.

Chapter 2

Exposure to actual physical violence, but not threatened physical violence alone, is associated with preadolescent brain morphology.

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Abstract

Background

Child behavior problems impose significant public health challenges. Higher quantities of adverse childhood experiences (ACEs)—regardless of type—increase the risk of child behavior disorders. Yet, brain development is sensitive to qualitative (not just quantitative) differences in adverse experiences. Researchers have investigated general measures of violence exposure as ACEs, but whether actual violence impacts brain structure differently than mere threatened violence alone has not been thoroughly assessed. This study tested our hypotheses that both actual and mere threatened violence exposure in childhood would be associated with smaller global and corticolimbic brain volumes in preadolescence, which have previously been associated with increased behavior problems. We further hypothesized that the magnitude of effects associated with actual violence exposure would be greater than those associated with mere threatened violence exposure.

Methods

We analyzed data from 2,905 children in the Generation R Study in Rotterdam, the Netherlands. When children were aged 10.1 years, study staff scanned children with magnetic resonance imaging (MRI) and asked mothers whether their child had ever experienced actual physical violence or threatened (but not actual) physical violence. We computed standardized global (total cortical, total subcortical, total white matter) and corticolimbic (hemisphere-averaged amygdala; hippocampus; rostral and caudal anterior cingulate cortex; lateral and medial orbitofrontal cortex) volumes, and we used OLS models to examine these outcomes adjusting for a range of relevant covariates.

Results

In fully adjusted models, actual violence exposure was associated with smaller volumes in all three global measures (e.g., total cortical gray volume β = -0.14, 95% CI: -0.26, -0.02). It was also associated with smaller amygdala volume (β = -0.17, 95% CI: -0.31, -0.04). However, we found no evidence that actual violence exposure was associated with any other corticolimbic volumes, nor did we find evidence that mere threatened violence alone was associated with any global or corticolimbic volume tested.

Conclusion

Actual violence exposure—but not mere threatened violence alone—may induce gray matter volume differences in preadolescence. These findings may have implications for programs designed to prevent childhood behavior problems.

Introduction

Childhood mental disorders, including behavior problems, impose significant short- and long-term impacts on the public's health, and they account for more medical spending in the United States ($13.9 billion in 2012) on children than any other condition.[1,2] Ample evidence demonstrates adverse childhood experiences (ACEs) increase the risk of child behavior problems.[63,64] Research on ACEs often assumes a dose-response relationship between accumulating adversities—regardless of type—and the likelihood of exhibiting behavior problems.[65] Brain development, however, is sensitive to qualitative differences in exposures.[28] ACEs models that count only the quantity and not the quality of adversities are therefore poorly suited to study how the social environment may influence neurodevelopment. This is problematic because a clear understanding of how the social environmental affects brain development may enable more effective public health interventions to disrupt socially toxic exposures, reduce the incidence of child behavior problems, and enhance child wellbeing. To address this challenge, emerging neurodevelopmental research has attempted to identify classes of adversities likely to influence brain health differently.[26,27] This strategy is promising because different classes of harmful exposures often but not always co-occur, and their effects on health may not be identical. As a result, some risk factors are likely more relevant than others to a given neurodevelopmental outcome.

One prominent model proposed by Sheridan and McLaughlin (2014) draws heavily on animal models and human behavioral research to suggest two classes of adverse experiences expected to impact brain development differently: (1) experiential deprivation, or the absence of expected cognitive and social input, and (2) physically threatening experiences.[25] Borrowing from the DSM-V definition of "traumatic event," they define threatening experiences as those

"characterized by *actual or threatened* . . . harm to one's physical integrity" (emphasis added).[25] Subsequent published research has generally supported their hypotheses that experiences of threat and deprivation have differential neurodevelopmental effects.[28] Sheridan and McLaughlin (2014) developed their key concepts based on evidence and definitions drawn from the dominant paradigms used in the fields of psychiatry and neuroscience. However, other social scientific fields concerned with the study of human behavior offer their own taxonomies of adversity-related concepts that may provide alternative classifications or at least further refine the definitions they advance. For example, since at least the 1700s, most legal systems have distinguished between physically threatening experiences in which the perpetrator actually strikes or touches the victim (i.e., "battery," hereafter referred to as "actual violence") and those in which the perpetrator threatens but does not actually strike, touch, or inflict injury on the victim (i.e., "assault," hereafter referred to as "mere threatened violence").[66] Within a legal context, this distinction between actual and mere threatened violence is informed by centuries of thinking about differences between specific behaviors and the consequences they are likely to cause. But within a neurodevelopmental context, this distinction has not yet been tested. Our study seeks to explore this knowledge gap.

Empirical research in both animals and humans has identified specific regions within the corticolimbic circuit of the brain that are reliably involved in processing threatening environmental stimuli. Thus, threat exposure has generally been associated with volumetric reductions in the amygdala, hippocampus, anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC).[20] However, testing whether these regions respond differently to actual versus mere threatened violence is difficult. Most neuroimaging studies of adversity in humans rely on case-control designs where cases are likely exposed to both actual and mere threatened violence, or

where they experience a more acute level of adversity than is prevalent in the population. Moreover, such studies tend to be limited by sample size and statistical power, which reduces their ability to detect differences in brain development caused by frequently co-occurring exposures. To overcome these limitations, this study draws on population neuroscience methods to leverage a large, population-based sample of children with differential exposure to actual violence, mere threatened violence, or both.

This study used data from the Generation R birth cohort, a population-based study tracking child development from pregnancy through adolescence. When participating children were about 10 years old, study staff collected retrospective data from mothers on their child's history of exposure to actual and threatened violence, and the children completed an MRI brain scan. Given that human cognition entails coordinated activity across many regions of the brain, we hypothesized that exposure to both actual violence and mere threatened violence would have global effects in the brain. Specifically, we hypothesized both exposures would be associated with reductions in three global measures of brain structure—(1) total cortical gray matter volume, (2) total subcortical gray matter volume, and (3) total white matter volume—even after extensive adjustment for plausible confounders selected based on findings from other studies. Critically, however, we also hypothesized that actual violence exposure would be associated with larger volumetric reductions than mere threatened violence exposure due either to the greater severity of the threat posed by the former or to qualitative differences between the two types of experiences. These associations would manifest, we postulated, in corticolimbic regions of the brain—i.e., the amygdala, hippocampus, rostral and caudal anterior cingulate cortex, and lateral and medial orbitofrontal cortex—given the corticolimbic circuit's role in threat detection and processing.

Methods

Participants.

This study uses data from the Generation R Study, a prospective, population-based birth cohort in Rotterdam, the Netherlands, seeking to identify social, environmental, and genetic factors affecting child health and development.[49] 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 all participants, study administrators have collected data from children and their caregivers at multiple time points through the present. All consent forms and study protocols are approved by the Medical Ethics Committee of the Erasmus Medical Center.

When participating children reached pre-adolescence (mean age 10.1 years, range 8.6 to 12.0 years), trained researchers at the Generation R research center in Rotterdam interviewed each child's primary caregiver, 96% of whom were mothers, about whether their child had been exposed to threatened or actual physical violence at any point in his or her childhood.[67] At the same study center visit, study staff scanned participating children with magnetic resonance imaging (MRI).[67] Primary analyses in the current study included participants if they had a usable MRI scan (described below) and reliable data from their mother on exposure to both mere threatened and actual violence. Among these participants, we excluded those whose mothers reported using cocaine or heroin while pregnant. Because some twins and triplets are enrolled in Generation R, we excluded all but one randomly selected sibling to avoid challenges with correlated data. Our final analysis sample included 2,905 children. See Supplement Section 2.1.1 for more information on selection into our analysis sample.

Measures.

This study drew on information obtained across the participants' lifetime regarding instances of actual and mere threatened violence from three different measurement instruments. Two of these measurement instruments assessed instances of harsh parenting and corporal punishment of children by their parents. These instances may have entailed experiences qualitatively similar to those of actual or mere threatened violence. Our hypotheses, however, are not confined to parent-perpetrated violence. Rather, they relate to any violence exposure regardless of perpetrator. Thus, our primary exposure uses information from a third measurement instrument; namely, from interview questions asking about actual and threatened violence exposure in the broadest terms. However, we used harsh parenting and corporal punishment data in secondary analyses to contextualize our primary analyses.

Violence Exposure

Actual and mere threatened violence exposure. During an in-person study center visit, study staff interviewed 5,354 mothers about their child's exposure to stressful life events. The interview adapted items from Kendler's Life Stress Interview and Brown and Harris' Life Event and Difficulty Schedule.[67–69] 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), two of which are germane to this study. English translations of the questions asked in Dutch are (1) "Has anyone ever used physical violence against your child, for example, beaten [him / her] up?" (hereafter referred to as "actual violence"); and (2) "Has anyone ever threatened to use physical violence against your child, such that it didn't happen but your child was scared?" (hereafter referred to as "mere threatened violence"). Interviewers marked responses as unreliable if

language barriers or other factors inhibited the mother's comprehension of the questions. We excluded these participants from our analyses.

Harsh Parenting. Generation R measured harsh parenting tactics used by mothers and partners separately with the Parent-Child Conflict Tactics Scale (CTS) via postal questionnaire.[70] When their children were aged 3.1 years (range 2.8 to 4.3), 4,733 mothers completed the CTS, 3,481 of whom later completed the stressful life events interview with Generation R staff when their children were aged 10.1 years. Parents reported how often they engaged in 3 types of verbally harsh and 3 types of physically harsh disciplinary tactics during the preceding two weeks on a six-point frequency scale ranging from "never" to "more than 4 times".[71] English translations of representative items administered in Dutch included "I shook my child," "I threatened to give a slap but I didn't do it," and "I called my child stupid or lazy or something like that." We constructed a continuous sum score ranging from 0 to 30 to quantify overall harsh parenting exposure for each child participant. We also constructed separate continuous sum scores ranging from 0 to 15 using three items corresponding to verbally harsh disciplinary tactics (hereafter called "verbal abuse") and three items corresponding to physically harsh disciplinary tactics (hereafter called "physical abuse").

Corporal Punishment. When participating children were 8.1 years old (range 7.5 to 10.0), 4,654 mothers completed a postal questionnaire containing 41 items from the Alabama Parenting Questionnaire (APQ), 2,701 of whose children later completed an MRI scan in preadolescence. 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".[72,73] 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.

Brain Imaging

Generation R researchers have described magnetic resonance imaging collection protocols and preprocessing pipelines elsewhere.[50] All scans were acquired by a GE MR-750W scanner (General Electric Healthcare, Chicago, IL) at 3T with an 8-channel head coil. An Inversion Recovery Fast Spoiled Gradient Recalled (IR-FSPGR) scanning sequence yielded 1 mm isotropic resolution. Study staff processed resulting images in FreeSurfer v6.0.0 using the Desikan-Killiany gyral-based cortical atlas. This process automatically produced both whole-brain volumes and subcortical volumes for several cortical and subcortical regions of interest (ROIs) in each hemisphere. Thereafter, study researchers visually inspected each reconstruction for quality control purposes and excluded poor quality images.

Our analyses used a selection of both global and ROI volumes (all in mm^3) based on our hypotheses. We assessed three global metrics, including (1) total cortical gray matter volume (all cortical tissue between the pial and white matter surfaces), (2) total subcortical gray matter volume (sum of volumes for the thalamus, caudate, putamen, pallidum, hippocampus, amygdala, and ventral diencephalon), and (3) total cerebral white matter volume (white matter tissue inside the white matter surface, excluding cerebellar white matter and the brainstem). ROIs included the amygdala, hippocampus, rostral and caudal anterior cingulate cortex (ACC), the lateral and medial orbitofrontal cortex (OFC).

Covariates

Researchers retrieved child birthdate and sex data from birth records. Parents self-reported the following: their country of origin and ethnicity, which we used to categorize child ethnicity as non-Turkish European (including Dutch), Turkish, Moroccan, 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; and parental prenatal psychopathology symptoms assessed using the 53-item Brief Symptom Inventory (BSI).[58] We calculated continuous BSI sum scores for each parent.

In all models, we multiply imputed missing covariate (but not exposure or outcome) data using chained equations to construct 50 imputed datasets, and we combined them using Rubin's Rules.[59] See Supplement Section 2.2 for additional details on our imputation models.

Statistical Analyses.

We excluded any participant with any outlying global or ROI volume 4 or more standard deviations from the measure's mean in the analysis sample (n = 14 excluded). Because we did not hypothesize hemisphere-specific effects, we averaged hemisphere-specific values for all ROIs and standardized all measures. We used t-tests to assess whether exposure and outcome values differed by important sociodemographic variables. We also calculated correlation coefficients for between actual violence exposure, mere threatened violence exposure, harsh parenting scores, and corporal punishment scores.

In primary analyses, we used ordinary least squares (OLS) linear regression to test whether exposure to actual and mere threatened violence were associated with continuous measures of brain morphology. First, we examined the three global outcomes. Where results from initial models suggested the presence of a global effect, we subsequently fit ROI-specific models to evaluate whether corticolimbic morphology partially explained the global effect. For each outcome, we fit (1) minimally adjusted models accounting for each child's age at MRI scan, sex at birth, and ethnicity; and (2) fully adjusted models incorporating the remaining covariates listed above, i.e., for parental income and education, parental psychosis history and psychopathology symptoms, child in utero exposure to smoking, and maternal age at childbirth. Next, because intracranial volume can affect subcortical volume, we adjusted both primary exposure-subcortical ROI models for intracranial volume to explore whether subcortical volumes differed over and above the global effects. In secondary analyses, we considered associations between the harsh parenting and corporal punishment scales and ROI outcomes in fully adjusted models. We focused both secondary analyses and sensitivity analyses on ROI outcomes (and not global outcomes) because we sought to gain clearer insight into specific regions of the brain possibly affected by our exposures of interest.

We conducted several sensitivity analyses to assess the extent to which our results were stable across different modeling strategies, specifications, and sample constructions. First, we fit marginal models of both primary exposures and all ROI outcomes using both (1) inverse probability of exposure weights and (2) standardization via the parametric G-formula.[74] These models attempt to estimate population average exposure effects—as opposed to the estimates of effects conditional on covariates obtained in our primary models—and thus require a somewhat different set of assumptions. By modeling our associations of interest in multiple ways, we gain

additional information about whether and to what extent our results may be robust to these different assumptions. See Supplement Sections 2.3 and 2.4 for more information regarding these models.

Second, to explore whether considering actual and mere threatened violence exposure together would change resulting effect estimates, we fit fully adjusted OLS models of all ROI outcomes that included covariates for both primary exposures simultaneously. Third, to further isolate the effects of actual versus mere threatened violence exposure, we fit fully adjusted OLS linear regression models for all ROI outcomes in subsamples excluding participants reporting both primary exposures, e.g., in models of actual violence exposure, we excluded participants reporting exposure to both actual violence and mere threatened violence. Finally, to explore whether effects of subtypes of harsh parenting might differ, we tested ROI effects in relation to physical abuse and verbal abuse harsh parenting subscales separately.

Results

Analytic sample characteristics.

The demographic characteristics of our primary analysis sample differed from those of the larger cohort at baseline. Included versus excluded participants were more likely to have non-Turkish European ethnicity (70% vs. 58%); to have parents with post-secondary educations (61% vs. 44%); to be from higher income households (50% vs. 32%); and to have older mothers (mean maternal age at birth 31.6 vs. 29.8 years).

Of 2,905 participants in our primary analytic sample, mothers reported that 202 children were exposed to actual violence (Table 2.1). The following groups were less likely to have been exposed to actual violence: girls versus boys (4.1% vs. 9.8%); children whose parents did versus

did not have a post-secondary education (5.6% vs. 8.8%); and children from higher versus lower income households (5.2% vs. 8.7%). Separately, mothers reported that 335 children were exposed to mere threatened violence. The following were less likely to have been exposed to mere threatened violence: girls versus boys (8.3% vs. 14.9%); children of higher versus lower educated parents (9.7% vs. 14.0%); and children from higher versus lower income households (9.6% vs. 13.5%). 66 mothers reported that their children were exposed to both actual and mere threatened violence.

Table 2.1. Distribution of primary and secondary exposures by participant characteristics in the final analysis sample.

	Total		Actual		Mere Threatened		Harsh		Corporal	
	n	%	*n*	%	*n*	%	$\bar{x}$	*s*	$\bar{x}$	*s*
Total Sample	2905	100.0	202	7.0	335	11.5	2.8	3.0	0.6	1.0
Child biological sex										
Female	1472	50.7	61	4.1	122	8.3	2.7	2.9	0.5	1.0
Male	1433	49.3	141	9.8	213	14.9	3.0	3.1	0.7	1.0
Child race / ethnicity / country of origin										
Dutch / Other European	1985	69.6	123	6.2	218	11.0	2.6	2.8	0.5	0.9
Turkish	148	5.2	8	5.4	12	8.1	3.0	3.5	0.6	1.0
Moroccan	126	4.4	8	6.3	14	11.1	4.5	4.6	1.3	1.4
Surinamese	212	7.4	23	10.8	30	14.2	3.8	3.3	1.0	1.1
Other	382	13.4	32	8.4	56	14.7	3.7	3.6	1.0	1.3
Highest Household Education										
Less than high school equivalent	116	4.3	7	6.0	10	8.6	3.0	3.2	0.8	1.0
High school or intermediate vocational train	946	34.7	87	9.2	142	15.0	3.3	3.4	0.8	1.1
Adv. vocational training, bachelor's, or high	1666	61.1	93	5.6	162	9.7	2.6	2.8	0.5	0.9
Household Income										
€2200 / month or less	1442	49.6	126	8.7	195	13.5	3.2	3.5	0.8	1.1
More than €2200 / month	1463	50.4	76	5.2	140	9.6	2.6	2.7	0.5	0.9

1. This table is based on observed values for each characteristic and does not account for missing data.

2. Maternal-report harsh parenting score was assessed at mean child age 3 years and has a theoretical range of 0 to 30.

3. Maternal-report corporal punishment score was asssessed at mean child age 8 years and has a theoretical score of 0 to 8.

Actual violence exposure was moderately correlated with mere threatened violence exposure (r = 0.19) but not meaningfully correlated with either harsh parenting (r = .01) or corporal punishment (r = -0.02). See Supplement Section 2.1.7 for additional correlations among these exposures.

Primary and secondary analyses.

In fully adjusted models, actual violence exposure was negatively associated with all three global outcomes assessed: total cortical gray matter volume, total subcortical gray matter volume, and total white matter volume (Table 2.2). Effect estimate magnitudes were moderate. For example, the smaller total cortical volume associated with actual violence exposure was nearly 70% of the larger total cortical volume associated with having higher income parents (i.e., -0.14 vs. 0.20, respectively). In contrast, we found no evidence that mere threatened violence was associated with any global outcome (Table 2.2).

Table 2.2: Associations between childhood actual violence exposure, mere threatened violence exposure, and standardized whole-brain structural outcomes in pre-adolescence. n = 2,905.

	Minimally adjusted models			Fully adjusted models		
Actual Violence	β	95% CI	*p*	β	95% CI	*p*
Cortical Brain Volume	-0.23	(-0.36, -0.10)	< 0.001	-0.14	(-0.26, -0.02)	0.03
Subcortical Brain Volume	-0.22	(-0.35, -0.09)	< 0.01	-0.15	(-0.28, -0.02)	0.02
White Matter Volume	-0.22	(-0.35, -0.10)	< 0.01	-0.16	(-0.28, -0.03)	0.01
Mere Threatened Violence						
Cortical Brain Volume	-0.04	(-0.14, 0.07)	0.51	0.04	(-0.06, 0.13)	0.45
Subcortical Brain Volume	-0.03	(-0.14, 0.07)	0.52	0.02	(-0.08, 0.12)	0.73
White Matter Volume	-0.01	(-0.11, 0.09)	0.81	0.04	(-0.06, 0.14)	0.43

1. Minimally adjusted models include covariates for child age and sex.
2. Fully adjusted models include covariates for child age, sex, ethnicity, and in utero exposure to smoking, and parental highest household education, household income, history of psychosis, psychopathology symptoms, and maternal age at birth.

In fully adjusted analyses of specific ROIs, actual violence exposure was associated with smaller amygdala volume (Table 2.3; $\beta_{\text{actual violence/amygdala}} = -0.17$, 95% CI, -0.31, -0.04). With the possible exception of lateral orbitofrontal cortical volume ($\beta_{\text{actual violence/lateral OFC}} = -0.13$, 95% CI, -0.26, 0.00), we found no evidence of an association between actual violence exposure and any other ROI, nor did we find evidence of associations between mere threatened violence and any ROIs. In models further adjusting for intracranial volume, the relationship between actual violence exposure and amygdala volume was somewhat attenuated, i.e., $\beta_{\text{actual violence + ICV /amygdala}} = -0.11$ (95% CI, -0.23, 0.00). Supplement Section 2.1.6 reports additional ICV-adjusted model results.

Table 2.3: Associations between childhood actual violence exposure, mere threatened violence exposure, and selected standardized corticolimbic structural outcomes in pre-adolescence. n = 2,905.

	Minimally adjusted models			Fully-adjusted models		
Actual Violence	β	95% CI	*p*	β	95% CI	*p*
Amygdala Volume	-0.21	(-0.35, -0.08)	< 0.01	-0.17	(-0.31, -0.04)	0.01
Hippocampus Volume	-0.15	(-0.29, -0.02)	0.03	-0.10	(-0.24, 0.03)	0.14
Anterior Cingulate Cortex						
Rostral Volume	-0.13	(-0.24, 0.01)	0.07	-0.08	(-0.21, 0.06)	0.28
Caudal Volume	-0.01	(-0.15, 0.13)	0.88	0.02	(-0.12, 0.17)	0.74
Orbitofrontal Cortex						
Medial Volume	-0.15	(-0.29, -0.02)	0.03	-0.09	(-0.23, 0.04)	0.16
Lateral Volume	-0.20	(-0.33, -0.06)	< 0.01	-0.13	(-0.26, 0.01)	0.06
Mere Threatened Violence						
Amygdala Volume	0.01	(-0.10, 0.11)	0.92	0.04	(-0.07, 0.14)	0.48
Hippocampus Volume	0.03	(-0.08, 0.14)	0.56	0.07	(-0.03, 0.18)	0.18
Anterior Cingulate Cortex						
Rostral Volume	0.01	(-0.10, 0.12)	0.87	0.05	(-0.06, 0.16)	0.40
Caudal Volume	0.03	(-0.08, 0.15)	0.59	0.05	(-0.06, 0.17)	0.37
Orbitofrontal Cortex						
Medial Volume	0.06	(-0.05, 0.16)	0.29	0.10	(-0.01, 0.21)	0.06
Lateral Volume	-0.03	(-0.14, 0.08)	0.58	0.03	(-0.08, 0.13)	0.61

1. Minimally adjusted models include covariates for child age and biological sex.
2. Fully adjusted models include covariates for child age, sex, ethnicity, and in utero exposure to smoking, and parental highest household education, household income, history of psychosis, psychopathology symptoms, and maternal age at birth.

In secondary analyses of other violence-related experiences, higher levels of both maternal harsh parenting tactics and maternal corporal punishment practices were also associated with smaller amygdala volumes (Table 2.4). Higher corporal punishment scores were also associated with smaller volumes in the rostral and caudal anterior cingulate cortex and the medial and lateral orbitofrontal cortex.

Table 2.4: Associations between maternal-report harsh parenting and maternal-report corporal punishment exposure and standardized frontolimbic outcomes in preadolescence.

	Harsh Parenting at Age 3, n = 2,031			Corporal Punishment at Age 8, n = 2,701		
	β	95% CI	*p*	β	95% CI	*p*
Amygdala Volume	-0.02	(-0.03, -0.01)	0.01	-0.04	(-0.08, -0.00)	0.05
Hippocampus Volume	-0.01	(-0.03, 0.00)	0.08	-0.03	(-0.07, 0.01)	0.13
Anterior Cingulate Cortex						
Rostral Volume	-0.01	(-0.03, 0.00)	0.06	-0.08	(-0.13, -0.04)	< 0.001
Caudal Volume	-0.02	(-0.03, -0.00)	0.02	-0.10	(-0.14, -0.05)	< 0.001
Orbitofrontal Cortex						
Medial Volume	0.00	(-0.01, 0.02)	0.77	-0.06	(-0.11, -0.02)	< 0.01
Lateral Volume	0.00	(-0.01, 0.01)	0.98	-0.08	(-0.12, -0.04)	< 0.001

1. Harsh parenting is a sum score of responses to 6 items assessing frequency of harsh parenting tactics, range 0 - 30.
2. Corporal punishment is a sum score of responses to 2 items assessing frequency of spanking and slapping, range 0 - 8.
3. Fully adjusted models include covariates for child age, biological sex, ethnicity, and in utero exposure to smoking, and parental highest household education, household income, history of psychosis, psychopathology symptoms, and maternal age at birth.

Sensitivity analyses.

Marginal models constructed using both inverse probability weights and standardization produced results similar to those of our primary models, i.e., actual violence exposure was associated with smaller amygdala volume (Supplement Section 2.1.2 reports detailed findings). The point estimate of the actual violence-amygdala volume association in the IPW-based model (β_{IPW} = -0.22, 95% CI, -0.38, -0.07) was somewhat larger than that in the model using

standardization to obtain marginal estimates ($\beta_{G\text{-formula}}$ = -0.17, 95% CI, -0.31, -0.04]). In addition, actual violence exposure was weakly associated with hippocampal volume (β_{IPW} = -0.15, 95% CI, -0.29, 0.00) in IPW-based (but not standardization) models, and with lateral orbitofrontal cortical volume ($\beta_{G\text{-formula}}$ = -0.13, 95% CI, -0.26, 0.00]) in standardization (but not IPW-based) models.

In fully adjusted OLS models of ROIs simultaneously including both actual and mere threatened violence exposure as covariates, actual violence exposure ($\beta_{actual/amygdala}$ = -0.18) maintained a substantive association with amygdala volume, while mere threatened violence exposure did not (Supplement Section 2.1.3). The magnitude of this effect estimate was similar to the magnitude of the estimate obtained when only actual violence was included in the model. Separately, actual violence exposure was weakly associated with lateral orbitofrontal cortical volume ($\beta_{actual/lateral\ OFC}$ = -0.13, 95% CI, -0.27, 0.00) in a model containing both exposures, which again mirrored results from our primary model.

In the subsample of 2,570 participants that excluded participants exposed to mere threatened violence, actual violence exposure was negatively associated with amygdala volume but not with any of the other ROI volumes we considered (Supplement Section 2.1.4). The magnitude of the actual violence-amygdala volume effect estimate in this model was similar to that of the primary model, i.e., $\beta_{subsample}$ = -0.18, (95% CI, -0.33, -0.02] vs. $\beta_{full\ sample}$ = -0.17, (95% CI, -0.31, -0.04). In the subsample of 2,739 participants that excluded those exposed to actual violence, mere threatened violence was positively associated with medial orbitofrontal volume—a result not seen in any other similar model. We found no evidence of associations with other ROI volumes.

Last, higher physical abuse scores were associated with smaller volumes in the amygdala, rostral ACC, and caudal ACC (Supplement Section 2.1.5). In contrast, higher verbal abuse scores were associated with smaller amygdala volume but not with other ROI volumes.

Discussion

Our results partially support our hypothesis that while both actual and mere threatened violence entail threats to one's physical integrity, they may affect brain development—and specifically the development of threat-responsive corticolimbic regions of the brain—quite differently. Actual violence exposure was associated with less volume in all global measures of brain morphology tested, including total cortical volume, total subcortical volume, and total white matter volume. Effect sizes for all three global measures were comparable, moderate, and of roughly similar magnitude to those associated with being from a lower-income family in our sample. One implication of this finding is that the potential 'benefit' of being from a higher-income family may be attenuated among children exposed to actual violence. In contrast, we found no evidence of any association between mere threatened violence exposure and any global outcome.

In secondary and sensitivity analyses exploring whether differences in corticolimbic morphology may account for the global effects, our results differed among the ROIs tested. All models of actual violence exposure and amygdala volume resulted in similar estimates regardless of modeling strategy or sample composition. In contrast, estimates of associations between actual violence exposure and both lateral orbitofrontal and hippocampal volume were inconsistent across modeling strategies and samples both in terms of effect estimate magnitudes and standard errors. Separately, we found no evidence of an association between actual violence exposure and

either anterior cingulate cortical or medial orbitofrontal cortical volume, and no evidence of associations between mere threatened violence exposure and any ROI.

Estimates of the association between actual violence exposure and smaller amygdala volume were robust and consistent in all sensitivity analyses. Specifically, these estimates remained stable whether models included participants with co-occurring exposure to mere threatening experiences or excluded them, and whether models additionally adjusted for co-occurring mere threatening experiences or not. Moreover, our primary OLS estimates were similar in magnitude and variance to those from both marginal models. This is notable given that interpretations of OLS model results are conditional on covariates included in the model, while interpretations of marginal model results are not, i.e., marginal model results estimate the average association between actual violence exposure and amygdala morphology in the entire study population. In this way, the three different modeling strategies (OLS, marginal models using inverse probability weights, and marginal models using the standardization via the parametric G-formula) provide complementary information and, taken together, decrease the likelihood that our results are spurious due to model misspecification.

Our measure of actual violence exposure was designed to capture a broad spectrum of experiences, which could include everything from corporal punishment or physical abuse by a parent to bullying or fighting on a playground. In contrast, our measures of corporal punishment and harsh parenting captured a more specific subtype of experiences, and the harsh parenting scale further assessed instances of verbal abuse not captured by the other measures of violence exposure. Nevertheless, insofar as the corporal punishment and harsh parenting measures asked about instances of actual physical touching meant to be threatening (e.g., spanking, slapping, or shaking), they share many qualitative attributes of physical violence captured by our primary

exposure measure (actual violence exposure), and thus they enable a form of replication of our primary findings. This is particularly true because they are not statistically correlated with actual violence exposure in our sample. Indeed, results from both alternative measures (i.e., harsh parenting and corporal punishment) support our findings with respect to actual violence exposure and amygdala morphology. Increased levels of maternal corporal punishment reported at mean child age 8.1 years were associated with decreases in amygdala volume. Similarly, increased levels of maternal harsh parenting tactics reported at mean child age 3.1 years were associated with decreases in amygdala volume. Thus, results from all three measures—each assessed at a different time and potentially capturing a slightly different universe of physically violent experiences—converge on a central finding: actual violence exposure in childhood is associated with reduced amygdala volume in preadolescence in a population-based sample.

Our findings in this population-based sample are notable because they build upon prior studies of similar exposures and outcomes conducted in smaller clinical samples. Most prior studies have reported similar findings linking increased violence exposure to decreased amygdala volume, but, for a host of reasons, they may not be generalizable to a broader population. For example, clinical samples typically recruit participants with more acute or traumatic forms of violence exposure than those seen commonly in the general population. Clinical samples are also more vulnerable to selection bias than birth cohorts because recruiting children exposed to trauma can be challenging if parents are reluctant to enroll their trauma-exposed children. By using data from a birth cohort, we reduced the threat of selection bias common to many case-control neuroimaging studies, increased the generalizability of our findings, and extended earlier work to a population-based sample where violence exposure may

be less acute. Thus, our study provides novel context when untangling the complex response of the amygdala to threatening experiences.

The dominant cumulative-risk model of ACEs accommodates quantitative differences in adversity exposure but not qualitative differences. It generally assumes heterogenous exposures result in *homogeneous* effects. However, this study and others suggest heterogeneous exposures result in *heterogeneous* effects, at least within the context of neurodevelopment.[63] Beyond the ACEs model, this study also suggests some additional nuance may be warranted when applying the threat-and-deprivation model advanced by Sheridan and McLaughlin (2014).[25] Their model posits roughly homogenous effects within domains of heterogenous exposures, e.g., experiences of threat will have similar neurodevelopmental consequences, whereas experiences of deprivation may have a different set of similar neurodevelopmental consequences. Yet, our results imply effect heterogeneity even within domains, i.e., between two types of threatening experiences. Actual violence exposure was associated with differences in multiple global and ROI volumes, while mere threatened violence exposure was associated with none. This suggests the possibility of qualitative differences between the two threat-related exposures.

These differences may be merely a function of exposure severity. Perhaps both actual and mere threatened violence exposure effect the same regions of the brain in the same way, with the latter exposure simply being a less acute—and thus less impactful—manifestation of the former. This would be consistent with the hypothesis advanced by Hanson et al. (2015) and others that violence-related early-life stress may lead to smaller brain volumes (particularly in the amygdala) because exposure to increasingly acute stressful experiences over time may cause increased neuronal excitation resulting in cell death and thus volumetric decreases. However, if actual and mere threatened violence exposure differed only by stressor severity (and not in some

other qualitatively important way), at least some effect estimates for both exposures would likely share directionality, even if the absolute magnitudes were different. Yet, our results do not support this possibility. In fact, effect estimates for mere threatened violence exposure were almost exclusively in the opposite direction as those for actual violence exposure, though substantial uncertainty surrounded most of them. This does not suggest actual and mere threatened violence exposure truly cause opposite effects—or even that mere threatened violence exposure truly has no effect on corticolimbic morphology—but this facet of our findings nevertheless subverts the suggestion that both exposures differ only in severity and not in some other qualitatively important way.

Our study has limitations. Because data for our primary exposures and outcomes were collected at the same time, our study is effectively cross-sectional. Separately, mothers retrospectively reported their children's' lifetime exposure to actual and mere threatened violence when their children were 10 years old, which can induce bias. We addressed both of these limits in part using secondary analyses testing harsh parenting tactics and corporal punishment, which were assessed prospectively when children were 3 and 8 years old, respectively. In addition, our models do not explicitly account for exposure severity, frequency or duration of exposure, or age at first exposure, all of which may be salient to neurodevelopment. Our study is also limited by challenges inherent in large, population-based pediatric neuroimaging studies. Differential attrition in the cohort by important sociodemographic characteristics and by scan quality, which is also socially patterned, limits the study's generalizability, although our use of inverse probability weights to account for attrition helps to address this concern. Finally, as with all observational studies, confounding and reverse

causation may have biased our results. For example, aggressive behavior—and the brain morphology associated with it—may have induced violence exposure.

Our study also has significant strengths that derive primarily from our large, population-based sample. First, our sample was large enough to investigate two frequently co-occurring exposures (i.e., actual and mere threatened violence) and to isolate their possible effects. In addition, our sample was more likely to capture less severe forms of violence exposure than samples in which violence-exposed children are specifically recruited. We were also able to triangulate findings from multiple measures (violence exposure, harsh parenting, corporal punishment) assessed at different timepoints in the participants' lives. And we were able to employ a variety of modeling strategies to assess the robustness of our results. Finally, by using an objective outcome measure, we avoid the threat of common method bias found in many studies of child behavioral development that rely on exclusively on parent reports for both exposures and outcomes.[75]

Conclusion

In our population-based sample of 2,905 children, childhood actual violence exposure was associated with decreased global brain volumes in preadolescence. It was also associated with decreased amygdala volume, a result that was robust to multiple sample compositions and modeling strategies. In contrast, childhood exposure to mere threatened violence was not associated with any brain outcome. These results suggest that two types of ostensibly similar threatening experiences may have a different set of neurodevelopmental consequences despite sharing many qualitative characteristics in common. Future studies considering effects of pre-defined classes of exposures should consider the possibility of effect heterogeneity not only

between but also within exposure classes. When defining these exposure classes, alternative taxonomies of behaviors and experiences from other fields—including those from the law—may provide complementary classification criteria.

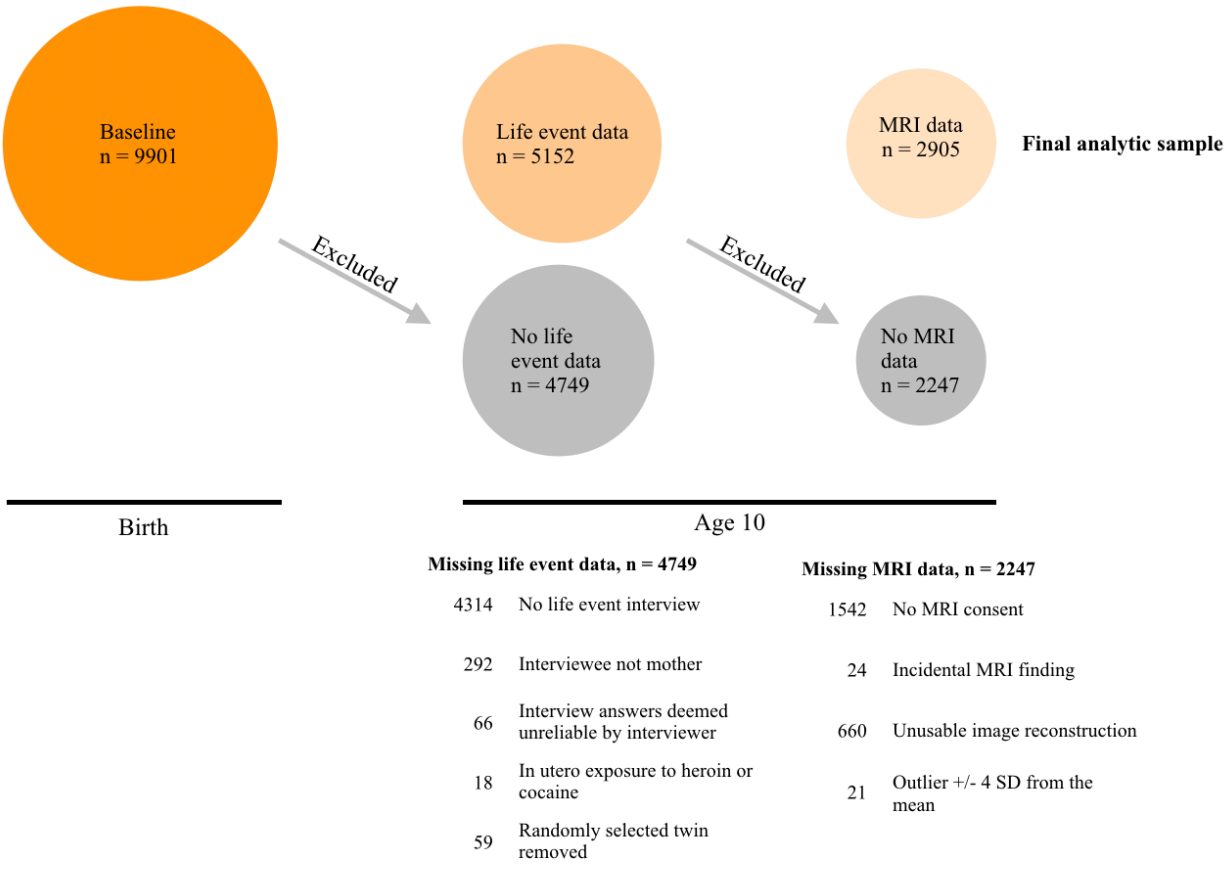
Our study contributes to research exploring how threatening experiences—and the childhood social environment more broadly—affect brain development, which in turn has important public health consequences. Violence exposure has been associated with increased child behavior problems in prior work. In clinical samples, research suggests this association is partially mediated by differences in amygdala function. Our findings extend this prior research beyond clinical samples to a population-based cohort, thereby strengthening the evidence base and providing additional context when untangling the complex neurodevelopmental and behavioral response to childhood violence exposure.

Appendix of Supplemental Information

Section 2.1. Supplemental figures and tables

Section 2.1.1. Sample composition.

Supplement Figure 1.



Supplement Table 1. 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.*

Section 2.1.2. Marginal models.

Supplement Section 2.1.2: Marginal models of associations between childhood actual violence exposure, exposure to mere threatened violence, and standardized corticolimbic outcomes in pre-adolescence; marginal models constructed using inverse probability of exposure weights. n = 2,905.

	Actual Violence Exposure			Mere Threatened Violence Exposure		
	β	95% CI	*p*	β	95% CI	*p*
Amygdala Volume	-0.22	(-0.38, -0.07)	< 0.01	-0.01	(-0.13, 0.12)	0.90
Hippocampus Volume	-0.15	(-0.29, 0.00)	0.05	0.04	(-0.07, 0.16)	0.46
Anterior Cingulate Cortex						
Rostral Volume	-0.06	(-0.22, 0.10)	0.46	0.04	(-0.09, 0.16)	0.56
Caudal Volume	0.03	(-0.12, 0.10)	0.68	0.05	(-0.09, 0.18)	0.48
Orbitofrontal Cortex						
Medial Volume	-0.09	(-0.24, 0.06)	0.24	0.04	(-0.09, 0.17)	0.57
Lateral Volume	-0.13	(-0.29, 0.03)	0.10	-0.03	(-0.16, 0.10)	0.64

Exposure probability models were fully adjusted and include covariates for child age, biological sex, ethnicity, and in utero exposure to smoking, and parental highest household education, household income, history of psychosis, psychopathology symptoms, and maternal age at birth.

Supplement Section 2.1.2: Marginal models of associations between childhood actual violence exposure, exposure to mere threatened violence, and standardized corticolimbic outcomes in pre-adolescence using standardization via the parametric g-formula. n = 2,905.

	Actual Violence Exposure			Mere Threatened Violence Exposure		
	β	95% CI	*p**	β	95% CI	*p**
Amygdala Volume	-0.17	(-0.31, -0.04)	0.01	0.03	(-0.08, 0.14)	0.65
Hippocampus Volume	-0.09	(-0.22, 0.04)	0.18	0.07	(-0.04, 0.17)	0.23
Anterior Cingulate Cortex						
Rostral Volume	-0.06	(-0.19, 0.08)	0.44	0.04	(-0.07, 0.16)	0.49
Caudal Volume	0.04	(-0.10, 0.18)	0.57	0.06	(-0.07, 0.18)	0.37
Orbitofrontal Cortex						
Medial Volume	-0.09	(-0.22, 0.04)	0.16	0.08	(-0.03, 0.20)	0.17
Lateral Volume	-0.13	(-0.26, 0.00)	0.05	0.01	(-0.10, 0.12)	0.88

All models are fully adjusted and include covariates for child age, biological sex, ethnicity, and in utero exposure to smoking, and parental highest household education, household income, history of psychosis, psychopathology symptoms, and maternal age at birth.
** P-values calculated after estimation based on bootstrap confidence intervals.*

Section 2.1.3. Models including covariates for both actual violence and mere threatened violence exposure simultaneously.

Supplement Section 2.1.3: Associations between actual violence exposure, mere threatened violence exposure, and standardized corticolimbic outcomes in preadolescence in models including both exposure variables simultaneously.

	Actual Violence Exposure			Threatened Violence Exposure		
	β	95% CI	*p*	β	95% CI	*p*
Amygdala Volume	-0.18	(-0.32, -0.05)	0.01	0.06	(-0.04, 0.17)	0.26
Hippocampus Volume	-0.12	(-0.26, 0.02)	0.09	0.09	(-0.02, 0.20)	0.11
Anterior Cingulate Cortex						
Rostral Volume	-0.09	(-0.23, 0.05)	0.22	0.06	(-0.05, 0.17)	0.22
Caudal Volume	0.01	(-0.13, 0.16)	0.85	0.05	(-0.07, 0.17)	0.39
Orbitofrontal Cortex						
Medial Volume	-0.12	(-0.25, 0.02)	0.09	0.12	(0.01, 0.22)	0.03
Lateral Volume	-0.14	(-0.27, -0.00)	0.05	0.05	(-0.06, 0.15)	0.41

Fully adjusted models include covariates for child age, sex, ethnicity, and in utero exposure to smoking; and parental highest household education, household income, history of psychosis, psychopathology symptoms; and maternal age at birth.

Section 2.1.4. Samples isolating primary exposures of interest, i.e., excluding participants with co-occurring actual and mere threatened violence exposure.

Supplement Section 2.1.4: Associations between actual violence exposure, mere threatened violence exposure, and standardized corticolimbic outcomes in preadolescence after excluding participants reporting both exposures.

	Actual Violence Exposure, n = 2,570			Threatened Violence Exposure, n = 2,739		
	β	95% CI	*p*	β	95% CI	*p*
Amygdala Volume	-0.18	(-0.33, -0.02)	0.03	0.06	(-0.05, 0.18)	0.29
Hippocampus Volume	-0.08	(-0.24, 0.08)	0.35	0.11	(-0.01, 0.23)	0.06
Anterior Cingulate Cortex						
Rostral Volume	-0.03	(-0.19, 0.14)	0.73	0.09	(-0.03, 0.21)	0.15
Caudal Volume	0.04	(-0.14, 0.20)	0.69	0.06	(-0.07, 0.19)	0.36
Orbitofrontal Cortex						
Medial Volume	-0.05	(-0.21, 0.10)	0.50	0.14	(0.03, 0.26)	0.02
Lateral Volume	-0.06	(-0.22, 0.10)	0.47	0.08	(-0.03, 0.20)	0.16

Fully adjusted models include covariates for child age, sex, ethnicity, and in utero exposure to smoking; and parental highest household education, household income, history of psychosis, psychopathology symptoms; and maternal age at birth.

Section 2.1.5. Models of harsh parenting subscales.

Supplement Section 2.1.5: Associations between dimensions of maternal-report harsh parenting exposure at age 3 and standardized corticolimbic outcomes in pre-adolescence. n = 2,031.

	Physical Abuse			Verbal Abuse		
	β	95% CI	*p*	β	95% CI	*p*
Amygdala Volume	-0.03	(-0.05, -0.00)	0.03	-0.03	(-0.05, -0.01)	0.01
Hippocampus Volume	-0.03	(-0.05, 0.00)	0.06	-0.01	(-0.04, 0.01)	0.22
Anterior Cingulate Cortex						
Rostral Volume	-0.03	(-0.51, -0.00)	0.03	-0.01	(-0.04, 0.01)	0.25
Caudal Volume	-0.03	(-0.06, -0.01)	0.02	-0.02	(-0.04, 0.00)	0.10
Orbitofrontal Cortex						
Medial Volume	0.01	(-0.02, 0.03)	0.62	0.00	(-0.02, 0.02)	0.95
Lateral Volume	-0.01	(-0.03, 0.02)	0.56	0.01	(-0.03, 0.03)	0.60

1. Physical Abuse scores are sums of responses to 3 items assessing frequency of harsh parenting tactics related to actual or threatened physical harm, range 0 - 15.
2. Verbal abuse scores are sums of responses to 3 items assessing frequency of harsh parenting tactics related to verbal abuse, range 0 - 15.
3. Fully adjusted models include covariates for child age, biological sex, ethnicity, and in utero exposure to smoking; parental highest household education, household income, history of psychosis, psychopathology symptoms; and maternal age at birth.
4. The correlation coefficient between the two measures is 0.49.

Section 2.1.6. ROI models additionally adjusting for ICV.

Supplement Section 2.1.6: Associations between childhood actual violence exposure and selected standardized subcortical structural outcomes in pre-adolescence with and without adjusting for estimated intracranial volume. n = 2,905.

	Fully-adjusted models			Fully-adjusted models + ICV		
	β	95% CI	*p*	β	95% CI	*p*
Amygdala Volume	-0.17	(-0.31, -0.04)	0.01	-0.11	(-0.23, 0.00)	0.05
Hippocampus Volume	-0.10	(-0.24, 0.03)	0.14	-0.02	(-0.14, 0.09)	0.68

** Fully adjusted models include covariates for child age, sex, ethnicity, and in utero exposure to smoking; parental highest household education, household income, history of psychosis, psychopathology symptoms; and maternal age at birth.*
** ICV = total intracranial volume as estimated by FreeSurfer v6.0.0.*

Section 2.1.7. Pairwise correlation coefficients between measures of violence exposure.

Supplement Table 1.7. Correlations among primary and alternative measures of violence exposure.

	Actual Violence	Mere Threatened Violence	Corporal Punishment	Harsh Parenting
Actual Violence	1			
Mere Threatened Violence	0.19	1		
Corporal Punishment	-0.02	0.02	1	
Harsh Parenting	0.01	0.10	0.32	1

Section 2.2. Multiple imputation models.

We imputed missing covariate data. The proportion of missing data for most covariates was low (< 2% for most variables), with the exception of household income (22%), maternal psychopathology symptoms (23%), partner educational attainment (36%), and partner psychopathology symptoms (38%). We used the 'mi impute chained' function in Stata 16.0/MP to conduct multiple imputation by chain 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.[59]

Section 2.3. Construction of marginal models using inverse probability weights.

We used logistic regression to model the propensity of each 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 linear regression models consisting only of the respective exposure and outcome.

Section 2.4. 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. 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.[59]

Chapter 3

Neurobehavioral effects of childhood violence exposure: alteration by family functioning but not sex or parental socioeconomic status.

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Abstract

Background

Childhood violence exposure may cause behavior problems, but a healthy family environment may buffer these effects. However, neural pathways mediating violence exposure

and behavior problems remain poorly understood, as are neural mechanisms by which protective factors buffer effects of violent experiences. This study tested our hypotheses that childhood violence exposure would be associated with lower amygdala volume and more behavior problems. We also hypothesized that healthy family functioning would buffer effects of violence exposure on amygdala volume and behaviors. Finally, we hypothesized that amygdala volume differences would partially mediate effects of violence exposure on behavior problems.

Methods

We analyzed data from 3,154 children in the Generation R Study in Rotterdam, the Netherlands. When children were in preadolescence, mothers completed the McMaster Family Assessment Device to measure family functioning (range 0 -3) and reported on their child's history of violence exposure. Children received magnetic resonance imaging brain scans, and fathers completed the 119-item Achenbach Child Behavior Checklist (CBCL, range in sample 0 - 117) about their child's behavior problems. We standardized hemisphere-averaged amygdala volumes, and we used weighted OLS models to examine amygdala volume and behavior problem. We tested for interaction with family functioning score, and we tested amygdala volume mediation of the association between violence exposure and behavior problems.

Results

In fully adjusted weighted models, violence exposure was associated with a 9.71 (95% CI: 3.86, 15.55) unit increase in CBCL score and a 0.20 (95% CI: -0.37, -0.03) standard deviation decrease in amygdala volume. Family functioning, but not parental SES or sex, altered both of these relationships. For example, violence exposure among children of lower functioning families was associated with a greater increase in behavior problems ($\beta = 20.03$) than among

children of higher functioning families ($\beta = 6.59$). We found no evidence that amygdala volume partially mediated the association between violence exposure and behavior problems.

Conclusion

A healthy family environment may partially buffer the effects of violence exposure on preadolescent behavior problems, and it may alter the effects of violence on brain morphology. Future research should investigate neural mechanisms through which protective factors buffer the negative effects of violence exposure on child development.

Introduction

Childhood behavior problems are associated with adverse outcomes later in life, including poor academic performance, substance use disorder, suicide, and criminality.[3–5] Empirical studies report childhood violence exposure is associated with higher levels of behavior problems, particularly externalizing problems (e.g., aggressive and rule-breaking behaviors) for boys and internalizing problems (e.g., anxious or withdrawn behaviors) for girls.[76,77] Studies also report that these relationships are modified by elements of the childhood social environment. In particular, a healthy family environment characterized by supportive relationships may buffer the effects of violence on child behavior problems.[78] However, biological mechanisms mediating violence exposure and behavior problems remain inadequately understood, as are mechanisms by which protective factors buffer effects of violent experiences.

Some research on these potential mechanisms has considered neural pathways, focusing on key brain regions involved in threat response, including the amygdala. Most of these studies report that increased childhood violence exposure is associated with mean reductions in adolescent amygdala volume.[28,79] Separately, early evidence suggests certain elements of a healthy family environment—namely, maternal positive parenting practices—may mitigate the effects of early-life stressful experiences (including violence exposure) on the development of certain brain regions, but results with respect to amygdala structure remain inconclusive.[80–82] Whether more general measures of a healthy family environment, including overall family functioning, may also buffer effects of adverse exposures on brain development has not yet been explored. In turn, research assessing relationships between violence exposure, the family environment, neural pathways, and behavior problems is also sparse.

These prior studies investigating violence exposure and buffering factors, brain development, and behavioral outcomes provide important insight, but they have some limitations. Most have been conducted in clinical or case-control samples of limited size, which tend to recruit participants exposed to more severe levels of violence. These samples also differ widely by sociodemographic characteristics, making cross-study comparisons difficult. Such studies have also rarely assessed the role of protective factors in detail because they either lack data on such factors, lack sufficient participants exposed to both risk and protective factors, or are not large enough to assess potential effect modification. Moreover, most such studies of behavioral outcomes rely upon a single reporter (usually the child's mother) for both exposure and outcome data, increasing the risk of common method bias. Multi-informant studies, in which one reporter provides exposure data while a different reporter provides outcome data, avoid this potential bias.[75]

Large population-based samples—together with epidemiologic methods from population neuroscience used to analyze them—may overcome many of these limitations. Compared to case-control samples, population-based cohorts are more likely to contain participants with greater variability in violence exposure severity because participants are not recruited explicitly on the basis of their exposure status. Such samples are also better suited to assess possible risk and protective factors because, by virtue of their size, they are more likely to include participants from a broader spectrum of socioeconomic backgrounds and life experiences as measured by multiple reporters. This enables investigators to explore a wider variety of possible risk and protective factors than is possible in smaller case-control samples.

To explore mediation and moderation of the relationships between violence exposure, brain structure, and behavior problems, this study used data from the Generation R Study, a

population-based birth cohort tracking child development from pregnancy through adolescence. Study staff collected data on the children's history of violence exposure (maternal report), family functioning (maternal report), and behavior problems (paternal report) over the course of follow-up. The children completed MRI brain scans when they were about 10 years old. Based on findings from prior literature, we hypothesized that higher versus lower exposure to violence in childhood would be associated with (1) lower preadolescent amygdala volume and (2) more preadolescent behavior problems. We also hypothesized effect modification by sex and three proxies of different facets of the childhood family environment. Specifically, we hypothesized these effects would be lesser among girls (vs. boys), children of higher income households (vs. lower income), children of higher educated parents (vs. lower educated), and higher functioning families (vs. lower functioning). Finally, we hypothesized that the relationship between higher violence exposure and higher behavior problems would be mediated partly by lower amygdala volume. See Supplement Section 3.1 for a graphic illustrating these hypothesized relationships.

Methods

Participants.

We used data from the Generation R Study, a prospective population-based birth cohort in Rotterdam, the Netherlands, seeking to identify factors affecting healthy child development.[49] The Generation R Study enrolled 9,978 new mother-infant dyads living in Rotterdam between 2002 and 2006. After securing the participants' informed consent, study administrators have collected data from children and their caregivers at multiple time points through the present through interviews and clinic visits. All consent forms and study protocols were approved by the Medical Ethics Committee of the Erasmus Medical Center.

When participating children reached preadolescence (mean age 10.1 years), study staff conducted in-person interviews of each child's primary caregiver, 96% of whom were mothers, about whether their child had experienced physical violence at any point in his or her childhood (n = 5,152).[83] At the same study center visit, participating children completed a magnetic resonance imaging (MRI) brain scan (n = 2,905).[67] At about the same time, fathers were asked to complete a postal questionnaire about their child's behavior problems (n = 3,154; median time between behavioral assessment and MRI scan = 7.7 weeks). The current study included participants if they had reliable violence exposure data from their mother and either (1) father-reported child behavior data or (2) a usable MRI scan. We excluded children exposed in utero to cocaine or heroin (n = 18). We also excluded all but one randomly selected sibling in cases where twins or triplets were enrolled in Generation R (n = 59). To maximize sample sizes, we included participants in analyses of child behavior outcomes (n=3,154) or of amygdala volume (n=2,905) if they had relevant data on that particular outcome, even if they were missing data on the other one. See Supplement Sections 3.2.1 and 3.2.2 for more information on attrition from baseline and selection into our analytic samples.

Measures.

Violence Exposure

This study's measure of violence exposure is derived from an in-person interview of mothers (described below) about whether their child ever experienced an incident of actual physical violence at any time prior to preadolescence regardless of perpetrator. To contextualize this broad exposure and explore whether reported instances of violence were perpetrated by parents (e.g., as with child abuse) or by someone outside the home, we drew on multiple additional sources of data Generation R staff collected at different times in the participants' lives

regarding instances of harsh parenting tactics, corporal punishment, family conflict, and conflict with a non-family member. Supplement Section 3.3.1 illustrates when these violence-related measures were collected.

Actual violence exposure. During an in-person study center visit when children were mean age 10.1 years (range 8.6 - 12.0), study staff interviewed 5,354 mothers asking whether their child had ever experienced (yes, no) any of 24 stressful life events including a question about physical violence.[67] Specifically, the English translation of the question asked in Dutch is "Has anyone ever used physical violence against your child, for example, beaten [him / her] up?" (hereafter referred to as "actual violence exposure" or "battery"). The question did not ask who perpetrated the violence. Interviewers marked responses unreliable if language or other barriers inhibited the mother's comprehension of the questions. We excluded these participants from our analyses (n = 66).

Additional violence-related measures. At the same in-person study center visit when children were mean age 10.1 years, study staff also asked mothers about their child's lifetime exposure (yes, no) to either (1) ongoing conflict with someone in the home or (2) ongoing conflict with someone outside the home. Earlier in the participants' lives, Generation R staff administered two instruments to assess instances of (1) harsh parenting tactics and (2) corporal punishment. When participants were about 3.0 years old, mothers and fathers separately answered postal questionnaires asking how often they engaged in any of six parenting tactics (e.g., shaking or threatening to slap their child) during the preceding two weeks on a six-point frequency scale ranging from "never" to "more than 4 times".[70] Based on their responses, we constructed two separate continuous sum scores ranging from 0 to 30 to quantify each child's harsh parenting exposure from each parent. Five years later, when children were mean age 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."[72,73] We combined answers to these two items to construct a continuous sum score ranging from 0 to 8 to quantify each participant's exposure to corporal punishment. For more information about the additional items described here, see Supplement Section 3.3.2.

Brain Imaging

Generation R researchers have described magnetic resonance imaging collection protocols and preprocessing pipelines elsewhere.[67] Mean child age at brain scan was 10.1 years (range 8.6 - 12.0). All scans were acquired by a GE MR-750W scanner (General Electric Healthcare, Chicago, IL) at 3T with an 8-channel head coil. An Inversion Recovery Fast Spoiled Gradient Recalled (IR-FSPGR) scanning sequence yielded 1 mm isotropic resolution. Study staff processed resulting images in FreeSurfer v6.0.0, which automatically calculated left and right amygdala volumes (mm^3) for each participant. Thereafter, study researchers visually inspected each reconstruction for quality control purposes and excluded poor quality images.

Child Behavior Problems

When their children were mean age 9.7 years (range 8.6 – 12.4), 3,154 fathers (out of 5,152 invited to participate) completed via postal questionnaire the Achenbach Child Behavior Checklist (CBCL/6-18). The CBCL/6-18 is a dimensional inventory of child behavior problems for school-aged children validated in Dutch and many other populations.[84,85] Fathers responded on a 3-point frequency scale (never, sometimes, often) to 119 items asking how often their children engaged in certain problematic behaviors. Given the large number of items, 11.2% of participants in our analytic sample were missing data on up to 24 items (see below for more details about missing data). Prior work demonstrates the items reliably load onto two broad

subscales measuring externalizing or internalizing behaviors as well as eight smaller subscales measuring more specific domains of behaviors (e.g., anxious behaviors, aggressive behaviors).[84] We summed responses to all 119 items to create a total behavior problem sum score (possible range 0 – 238). Secondary analyses used continuous subscale sum scores for internalizing behaviors (possible range 0 – 64) and externalizing behaviors (possible range 0 – 70), as well as continuous sum scores for the two subdomains of externalizing behaviors: aggressive behaviors (possible range 0 – 36) and rule-breaking behaviors (possible range 0 – 34). Supplement Section 3.3.3 provides more detail on the CBCL subscales.

Potential Moderators

Family functioning. Mothers completed via postal questionnaire the McMaster Family Assessment Device, General Functioning Subscale to assess family functioning when their children were mean age 9.7 years (range 8.7 – 12.0).[86,87] Prior work demonstrates this self-report survey is both reliable and valid in Dutch populations.[88] Mothers responded on a 4-point Likert scale to six positively framed and six negatively framed items about their family environment (e.g., "If there are problems, we can count on each other for support," and, "There are a lot of unpleasant and painful feelings in our family"). Because questions do not reference specific family members or roles, mothers can respond regardless of their family's structure. We derived a continuous family functioning score by reverse-scoring negatively framed items, then averaging response scores across all 12 items. This yielded a family functioning score range of 0 to 3 for each participant, where higher scores indicated more positive functioning. Cronbach's alpha reliability coefficient in the analytic sample was strong (0.90). We also constructed a binary measure ($<$ or ≥ 2.0) drawing on *a priori*-defined considerations of the scale's response options for use in sensitivity analyses.

Socioeconomic status. At study enrollment, parents self-reported their household income (< or ≥ €2200 / month) and their highest completed education level (less than high school equivalent; high school or intermediate vocational training; advanced vocational training, bachelor's degree, or higher). We derived a household education measured as the highest level of education achieved by either parent.

Sex. Researchers retrieved child sex data from birth records.

Additional Covariates

Researchers retrieved participants' birthdates from birth records. As study enrollment, parents self-reported the following: their country of origin and ethnicity, used to categorize child ethnicity as non-Turkish European (including Dutch), Turkish, Moroccan, and Other Ethnicity; maternal and paternal history of psychotic episodes (yes / no for each parent); maternal age at childbirth; maternal smoking history during pregnancy (never, until pregnancy was known, or through pregnancy); and parental prenatal psychopathology symptoms assessed using the 53-item Brief Symptom Inventory (BSI).[58] We calculated continuous BSI sum scores for each parent.

Missing Data

For behavioral outcomes, we included participants with data on at least 80% (95 items) of the 119 items of the CBCL. In our behavioral outcome analysis sample (n = 3,154), 88.8% of participants were missing no CBCL data, and another 7.5% were missing data on only 1 of the 119 items. For brain structural outcomes, we included only participants with no missing data. We multiply imputed missing CBCL and covariate data (but not missing data on amygdala volume) using chained equations to construct 50 imputed datasets. Then, we combined imputation-

specific mean and variance measures for each imputed variable using Rubin's Rules.[59] See Supplement Section 3.4.1 for details about our imputation models.

Separately, to address possible bias resulting from differences in sample composition for behavioral, brain structural, and mediation models, we used inverse probability of attrition weights (IPWs) in all analyses. When calculating the IPWs, we deemed as lost to follow-up any participant enrolled at the Generation R baseline but excluded from the relevant analytic sample for any reason. Supplement Section 3.4.2 contains additional details regarding IPW construction.

Statistical Analyses

Family functioning may have a very different effect or interpretation as a moderator of violence exposure if the violence to which children are exposed is perpetrated by a family member rather than a non-family member. To understand whether the perpetrators of violence were family members or non-family members, we compared levels of harsh parenting practices, corporal punishment, and family vs. nonfamily conflict (described above) between children identified as violence-exposed or violence-unexposed per our primary exposure measure. First, we assessed differences in mean maternal and paternal harsh parenting scores and mean corporal punishment scores between violence-exposed versus violence-unexposed children using two-sample t-tests assuming equal variances. Next, we compared the odds of experiencing either ongoing conflict in the home or ongoing conflict outside the home among violence-exposed vs. -unexposed children by calculating odds ratios.

To assess the association between childhood violence exposure and total behavior problems, we fit two models using ordinary least squares (OLS) linear regression: (1) a minimally adjusted model adjusting for child sex, ethnicity, and age at outcome; and (2) a fully

adjusted model including covariates in minimally adjusted models plus parental education, income, psychopathology symptoms, and history of psychosis, child in utero smoking exposure, and maternal age at birth. To evaluate if sex, parental education, parental income, or maternal family functioning score (continuous and binary) modify the relationship between childhood violence exposure and total behavior problems, we fit separate fully adjusted OLS models including a violence exposure-by-moderator interaction term. In the event a categorical interaction term was significant, we stratified our analyses. We weighted all models to account for attrition from baseline using IPWs.

In secondary analyses, we explored associations between violence exposure and behavior problem subtypes following a similar modeling strategy. First, we examined violence exposure in relation to the CBCL externalizing and internalizing behavior subscales in fully adjusted models. We tested whether those associations were altered by the same potential moderators tested above by adding violence exposure-by-moderator interaction terms. If results suggested moderation of the association between violence exposure and either the externalizing or the internalizing behavior subscale, we further evaluated each subscale's subcomponents (e.g., for externalizing behaviors, we tested the aggressive behavior and rule-breaking behavior subcomponent scales) in relation to violence exposure, and we also considered potential effect modification as described above.

In analyses of amygdala volume, we excluded participants with outlying left or right volumes ≥ 4 standard deviations from the sample mean (n = 14 excluded). Because we did not hypothesize hemisphere-specific effects, we summed left and right hemisphere volumes, then standardized the resulting measure within our analysis sample. To assess the association between violence exposure and amygdala volume, we fit both minimally and fully adjusted OLS linear

regression models using the same sets of covariates used for models of behavior problems. Next, we tested for moderation of the violence exposure-amygdala volume relationship by sex, parent education, parent income, and maternal family functioning score (continuous and binary) by including a violence exposure-by-moderator interaction term in separate fully adjusted models for each potential moderator. All models included IPWs to account for attrition from baseline.

Finally, to examine whether differences in amygdala volume explained any of the association between violence exposure and total problem behaviors, we used bootstrapped regression models to estimate (1) the total effect of violence exposure on total behavior problems; (2) the direct (unmediated) effect; and (3) the indirect effect, i.e., the effect of violence exposure on total behavior problems explained by differences in amygdala volume.[89] Each of these models was fully adjusted for covariates described above. Supplement Section 4.3 provides more detail on these models. In secondary analyses, we stratified our mediation models by factors previously identified as possible moderators of the relationship between violence exposure and total behavior problems.

Results

Characteristics of analytic samples

Demographic characteristics of the unweighted analytic samples differed from those of the full cohort at baseline (Supplement Section 3.2.2). Among individuals included in analyses of behavioral outcomes, included vs. excluded participants were more likely to be of non-Turkish European ethnicity (81% vs. 51%), from higher vs. lower income households (61% vs. 26%), and to have parents with vs. without post-secondary educations (67% vs. 33%). Differences were

similar (albeit of smaller magnitude) in the amygdala volume analysis sample. (Supplement Section 3.2.2).

Of 3,154 participants in the behavior problem sample, mothers reported 190 children exposed to actual violence (Table 3.1). Boys were more likely than girls to have been exposed (8.7% vs. 3.5%), as were children from lower vs. higher income households (7.4% vs. 5.2%) and children whose parents did not vs. did have a post-secondary education (7.4% vs. 5.4%). We found similar patterns of exposure in our amygdala volume sample (Table 1). Family functioning scores were relatively high (mean = 2.6, range 0 – 3) and left skewed, i.e., 89% of participants had scores of 2.0 or higher (Supplement Section 2.3). The mean total behavior problem score in our behavior analysis sample was 17.3 (sample range 0 – 117). On average, boys had higher scores than girls (19.0 vs. 15.6), indicating boys exhibited more behavior problems. Children of lower vs. higher income households and of parents without vs with a post-secondary education also had higher total problem scores and smaller amygdala volumes. See Supplement Section 3.2.3 for more detail.

Table 3.1. Distribution of violence exposure and participant sociodemographic characteristics in primary analytic samples.

	Amygdala Volume Sample				Behavior Problem Sample			
	Total		Violence Exposed		Total		Violence Exposed	
	n	%	*n*	%	*n*	%	*n*	%
Total Analytic Sample	2905	100.0	201	6.9	3154	100.0	190	6.0
Child biological sex								
Female	1472	51.0	61	4.2	1607	51.0	56	3.5
Male	1433	49.0	140	9.8	1547	49.0	134	8.7
Child ethnicity / country of origin								
Dutch / Other European	1986	69.6	123	6.2	2541	81.0	149	5.9
Turkish	148	5.2	8	5.4	98	3.1	3	3.1
Moroccan	126	4.4	8	6.4	66	2.1	6	9.1
Surinamese	212	7.4	23	10.9	149	4.8	12	8.1
Other	381	13.4	31	8.1	283	9.0	20	7.1
Highest Household Education								
Less than high school equivalent	115	4.2	7	6.1	47	1.6	2	4.3
High school or intermediate vocational training	946	34.7	87	9.2	867	28.5	70	8.1
Adv. vocational training, bachelor's, or higher	1665	61.0	91	5.5	2128	70.0	114	5.4
Household Income								
€2200 / month or less	1441	49.6	125	8.7	1235	39.0	91	7.4
More than €2200 / month	1464	50.4	76	5.2	1919	61.0	99	5.2

See Supplement Section 3.2.3 for distributions of family functioning scores and outcome measures by participant sociodemographic characteristics.

The odds of mothers reporting that their child experienced ongoing conflict with a *family* member were 3.5 times greater for violence-exposed vs. non-violence-exposed children, while the odds that children were exposed to ongoing conflict with a *non-family* member were 5.6 times greater for violence-exposed vs. non-violence-exposed children. Separately, frequency of harsh parenting tactics (maternal or paternal) and corporal punishment did not differ by violence exposure status. See Supplement Section 3.2.4.

Associations between violence exposure and behavior problem measures

In a fully adjusted model, actual violence exposure was associated with a 9.71 (95% CI: 3.86, 15.55) unit increase in total behavior problem score (Table 3.2). See Supplement Section 3.5.1 for a comparison of results between minimally adjusted and fully adjusted models. Among the four possible moderators tested, only family functioning appeared to modify the association between violence exposure total problem behavior scores. Specifically, more positive family functioning attenuated the higher level of behavior problems associated with violence exposure. For example, in models using a binary (high / low) family functioning variable, the magnitude of the association between violence exposure and total behavior problem score was less than half as large among higher functioning families compared to lower functioning families (Table 3.2, Figure 3.1).

Table 3.2. Main effects and interaction estimates of the associations between maternal-report childhood actual violence exposure and two outcomes in preadolescence: amygdala volume and partner-reported total behavior problems.

	Amygdala Volume			Total Behavior Problems		
	β	95% CI	*p*	β	95% CI	*p*
Main effect (no interaction)						
Violence exposure	-0.20	(-0.37, -0.03)	0.02	9.71	(3.86, 15.55)	< 0.01
Interaction models						
Sex						
Violence exposure	-0.33	(-0.64, -0.02)	0.04	4.58	(-1.22, 10.37)	0.12
Violence exposure * Boy	0.19	(-0.17, 0.55)	0.30	6.92	(-2.53, 16.38)	0.15
Parent Income						
Violence exposure	-0.12	(-0.37, 0.12)	0.32	11.50	(2.06, 20.94)	0.02
Violence exposure * Higher income	-0.17	(-0.58, 0.23)	0.41	-4.29	(-16.51, 7.93)	0.49
Parent Education						
Violence exposure	-0.09	(-0.36, 0.17)	0.49	10.80	(1.61, 19.98)	0.02
Violence exposure * Parent education	-0.20	(-0.59, 0.19)	0.31	-2.68	(-16.19, 10.84)	0.70
Family Functioning (continuous)						
Violence exposure	1.00	(0.23, 1.76)	0.01	39.27	(12.63, 65.91)	< 0.01
Violence exposure * Functioning	-0.51	(-0.84, -0.19)	< 0.01	-13.14	(-23.80, -2.49)	0.02
Family Functioning (binary)						
Violence exposure	0.24	(-0.03, 0.53)	0.08	20.03	(7.65, 32.41)	< 0.01
Violence exposure * High functioning	-0.54	(-0.87, -0.21)	< 0.01	-13.44	(-27.50, 0.62)	0.06

1. All models are fully adjusted and include covariates for child age at outcome assessment, sex, ethnicity, and in utero smoking exposure; parental highest household education, household income, history of psychosis, and psychopathology symptoms; and maternal age at child's birth.

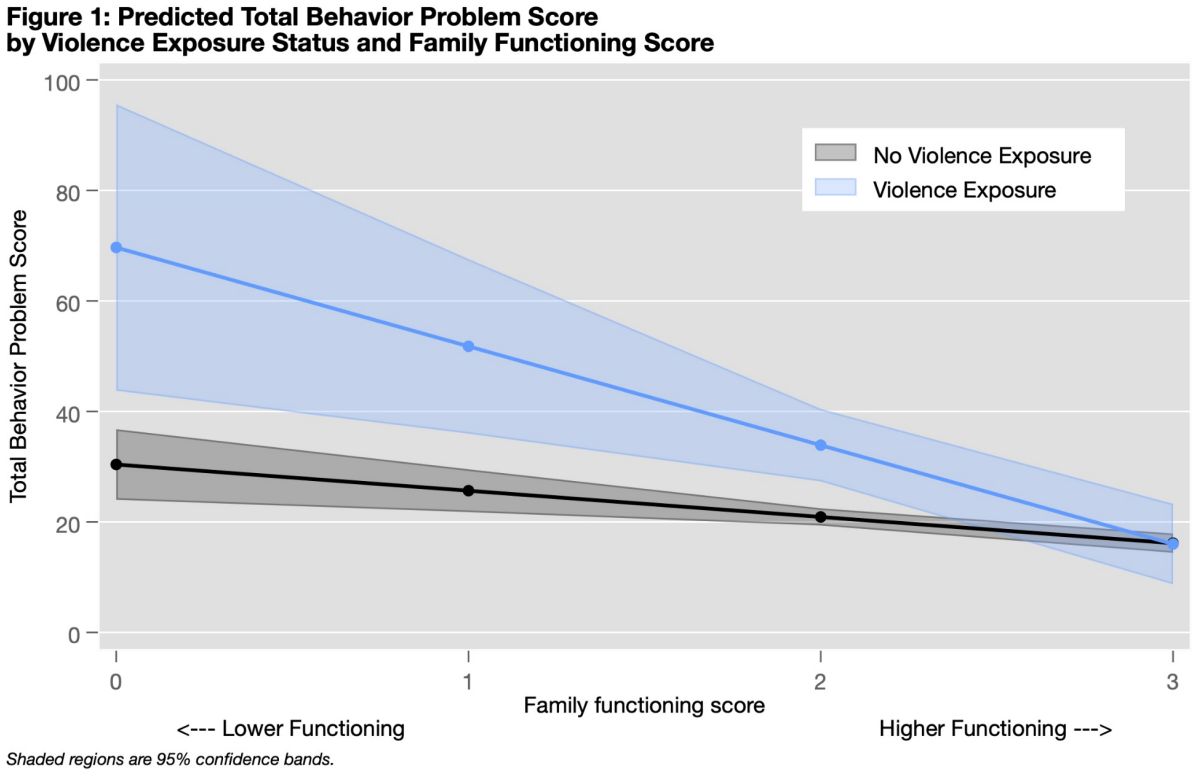
2. All estimates are weighted to reflect differential attrition from the baseline sample to the analysis samples; unweighted n for total behavior problems = 3,154; unweighted n for amygdala volume = 2,905.

3. The continous family functioning measure has range 0-3; the binary functioning measure is < or ≥ 2.0.

4. Total behavior problems are measured by the partner-reported CBCL total score; range in sample 0 - 117.

5. Amygdala volume is assessed as the standardized bilateral mean for each participant.

Figure 3.1



In secondary analyses, violence exposure was associated with higher levels of both internalizing (β = 2.47, 95% CI, 0.92, 4.02) and externalizing (β = 2.90, 95% CI, 0.83, 4.97) behavior problem scores in fully adjusted models. Family functioning modified the association between violence exposure and externalizing behaviors but not internalizing behaviors. Using a binary (high / low) family functioning variable, the estimated effect of violence exposure on externalizing behaviors was less than half as large among higher functioning families compared to lower functioning families (Table 3.3). Sex, parental income, and parental education did not alter relationships between violence exposure and either externalizing or internalizing behaviors (Supplement Section 3.5.3). In additional analyses considering the subcomponents of the externalizing behavior subscale, violence exposure was associated with aggressive behaviors (β = 2.58, 95% CI, 0.09, 4.26) but not rule-breaking behaviors. The magnitude of the aggressive

behavior effect estimate suggested the increase in aggressive behaviors accounted for most of the increase in externalizing behaviors associated with violence exposure. Finally, we found evidence of interaction by family functioning in the association between violence exposure and aggressive behaviors in a manner consistent with results above (Table 3.3).

Table 3.3. Main effect and interaction estimates of the associations between childhood violence exposure and behavior problem subtypes.

	Externalizing Behaviors									Internalizing Behaviors		
	Overall Externalizing Score			Aggressive Behavior Score			Rule-breaking Behavior Score			Overall Internalizing Score		
	β	95% CI	*p*	β	95% CI	*p*	β	95% CI	*p*	β	95% CI	*p*
Main effect (no interaction)												
Violence exposure	2.90	(0.83, 4.97)	0.01	2.58	(0.90, 4.26)	< 0.01	0.32	(-0.22, 0.85)	0.24	2.47	(0.92, 4.02)	< 0.01
Interaction models												
Family Functioning (continuous)												
Violence exposure	14.31	(4.17, 24.44)	0.01	12.31	(4.38, 20.24)	< 0.01	2.00	(-0.70, 4.69)	0.15	7.31	(-0.16, 14.78)	0.06
Violence exposure * Functioning	-5.06	(-9.07, -1.05)	0.01	-4.30	(-7.42, -1.19)	0.01	-0.75	(-1.83, 0.32)	0.17	-2.17	(-5.13, 0.78)	0.15
Family Functioning (binary)												
Violence exposure	7.43	(2.55, 12.32)	< 0.01	6.28	(2.67, 9.90)	< 0.01	1.15	(-0.33, 2.63)	0.13	3.88	(0.26, 7.50)	0.04
Violence exposure * High functioning	-5.89	-11.25, -0.53)	0.03	-4.80	(-8.85, -0.75)	0.02	-1.09	(-2.67, 0.49)	0.18	-1.86	(-5.87, 2.15)	0.36

1. All models are fully adjusted and include covariates for child age at outcome assessment, sex, ethnicity, and in utero smoking exposure; parental highest household education, household income, history of psychosis, and psychopathology symptoms; and maternal age at child's birth.

2. All estimates are weighted to reflect differential attrition from the baseline sample to the analysis sample; unweighted n for total behavior problems = 3,154.

3. The continous family functioning measure has range 0-3; the binary functioning measure is < or ≥ 2.0.

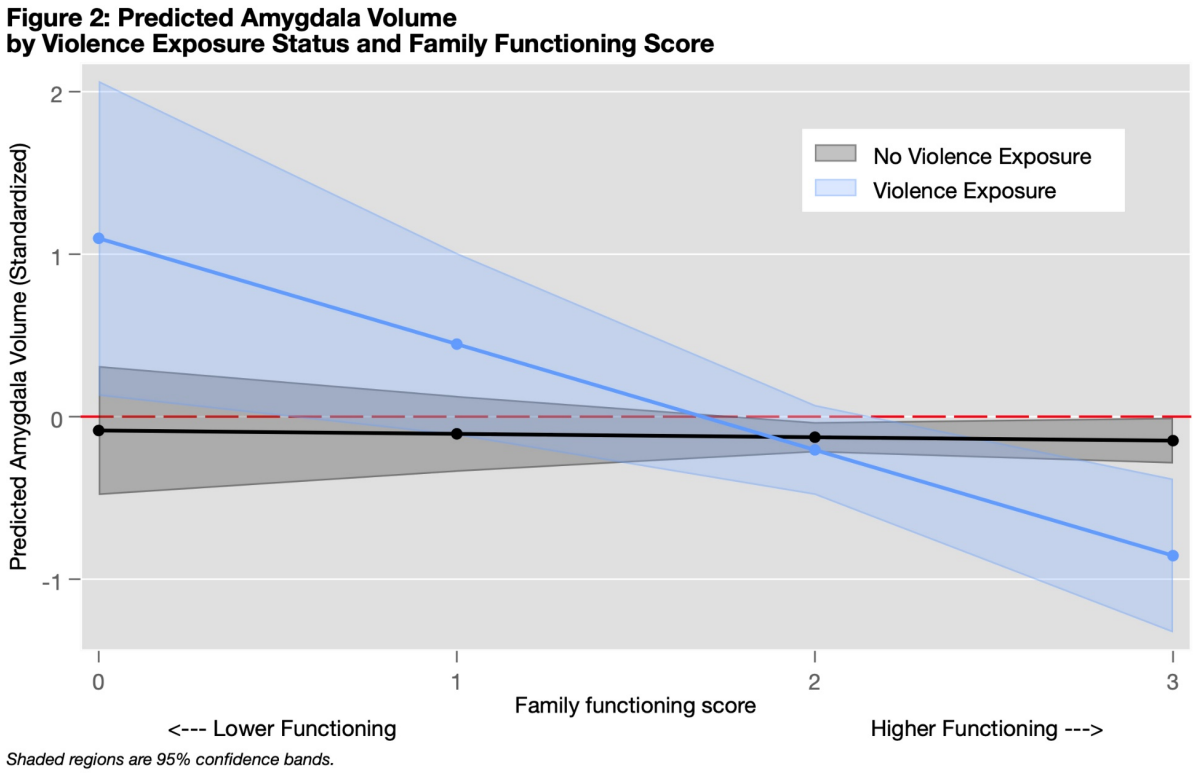
4. All outcome measures are continuous with the following ranges in this sample: internalizing behaviors 0 - 36; externalizing behaviors 0 - 48; aggressive behaviors 0 - 34; rule-breaking behaviors 0 - 16.

5. See Supplement Section 3.5.3 for results testing sex, parental income, and parental education as moderators. We did not test internalizing behavior subtypes for interaction because we found no evidence of interaction by our hypothesized moderators of the relationship between violence exposure and the overall internalizing behavior score.

Associations between violence exposure and amygdala volume

Violence exposed vs. unexposed children had a mean 0.20 standard deviation (95% CI, -0.37 -0.03) lower amygdala volume in a fully adjusted model (Table 3.2). See Supplement Section 3.5.2 for a comparison of results from minimally versus fully adjusted models. As with our behavioral analyses, we found evidence for interaction by family functioning. Models using both measures of family functioning (continuous and binary) produced similar results: exposed versus unexposed children from the lowest functioning families demonstrated *higher* amygdala volume, but exposed versus unexposed children from the highest functioning families demonstrated *lower* amygdala volume (Table 3.2, Figure 3.2). Subsequent models found no evidence for interactions by sex, parental education, or parental income.

Figure 3.2



Mediation analyses

Our fully adjusted mediation model estimated an indirect effect (i.e., effect mediated by differences in amygdala volume) of violence exposure on increased total behavior problems of 0.05 units (95% CI, -0.29, 0.40), compared to a total effect of a 9.30 unit (95% CI, 2.50, 16.10) increase in total problem score. This total effect point estimate differs slightly from results in Table 3.2 because mediation models used a different analytic sample and inverse probability weights. Models stratified by binary family functioning score ($<$ or ≥ 2.0) provided no additional evidence that amygdala volume mediated the association between violence exposure and total behavior problems among either low or high functioning families (Supplement Section 3.5.4).

Discussion

Our results suggest that childhood violence exposure affects both brain morphology and behavior in preadolescence in a population-based birth cohort, and that healthy family functioning in preadolescence may substantially buffer these effects. Three specific findings warrant note. First, childhood violence exposure was associated with increased preadolescent total behavior problems, including both internalizing and externalizing problems. The increase in externalizing behaviors appears to be due almost entirely to higher levels of aggressive behavior. Second, violence exposure was also associated with differences in amygdala volume. Third, healthy family functioning modified the relationships between violence exposure and both preadolescent amygdala volume and behavior problems. Estimated effects of violence exposure on problem behaviors among higher functioning families were less than half the magnitude of those among lower functioning families.

Because parents who use harsh or corporal punishment tactics are often the perpetrators of violence against children, the potential buffering role of family functioning warrants scrutiny. Our findings would be less plausible if parents were responsible for both violence exposure and family functioning. Generation R staff did not collect data on perpetrators of violence, but our descriptive analyses comparing rates of harsh parenting, corporal punishment, and both family and non-family childhood conflict mitigate concerns that our primary measure of violence exposure occurred in the home. For example, if parents were the primary perpetrators of violence against violence-exposed children, one might expect that harsh parenting or corporal punishment scores would be higher among violence-exposed children or that the odds ratio for family conflict would be higher than for non-family conflict among exposed versus unexposed children. However, the data do not support these expectations. Neither levels of maternal nor paternal harsh parenting tactics assessed when children were about 3 years old differed between violence-exposed versus -unexposed children. The same was true for levels of corporal punishment assessed when children were about 8 years old. Moreover, the odds of exposure to ongoing conflict with someone *outside* the family were substantially higher for violence-exposed versus -unexposed children than were the odds of exposure to ongoing conflict *within* the family. Taken together, these results suggest many perpetrators of violence against violence-exposed children in this sample were plausibly non-family members.

This study offers notable findings to the literature. For example, on *average* in our sample, violence exposure was associated with decreased amygdala volume, but this relationship was not uniform across levels of family functioning. Rather, violence exposure (versus no violence exposure) was associated with an *increase* in amygdala volume among lower functioning families but a *decrease* in volume among higher functioning families. However,

because more violence-exposed participants came from higher functioning families, the *average* effect in the sample suggested a *decrease* in volume. This suggests the family environment within which children experience violence may alter the directionality of the association between violence exposure and amygdala volume.

Our results are partially consistent with models and taxonomies of stressful experiences from prior literature that posit different (and sometimes opposing) neurobiological consequences depending on the severity and duration of stressor exposure. For example, Sapolsky (2015) and McEwen et al. (2015) review evidence suggesting mild-to-moderate stress induces adaptive (i.e., possibly beneficial) changes to the brain, while severe stress induces opposing, deleterious brain changes.[90,91] Similarly, Shonkoff, Boyce & McEwen (2009) differentiate "tolerable stress" (i.e., potentially deleterious stress buffered by supportive relationships) from "toxic stress" (i.e., extended exposure to unbuffered stressors), the latter of which—by their definition—disrupts healthy brain development.[92] Applied here, violence exposure buffered by a healthy family environment may cause mild-to-moderate (or "tolerable") stress leading to decreased amygdala volume, while unbuffered violence exposure may cause severe (or "toxic") stress leading to increased amygdala volume. This is also consistent with findings from rodent models in which chronic stress exposure causes increased dendritic spine density and arborization in the basolateral amygdala subregion, which may explain the increase in overall amygdala volume associated with unbuffered violence exposure.[93,94]

However, our results are also partially *inconsistent* with these models and taxonomies. For example, it is unclear that decreased amygdala volume is an adaptive or beneficial response to mild-to-moderate stress, or that increased amygdala volume is a maladaptive response to severe stress. In fact, several studies report that *decreased* amygdala volumes are associated with

increased child behavior problems, while others find that *increased* amygdala volumes are associated with increased child behavior problems.[95–99] Moreover, we are aware of no literature suggesting a mechanism by which mild-to-moderate stress induced by buffered violence exposure would lead to decreased amygdala volume. While most prior neuroimaging studies in humans report increased violence exposure is associated with decreased amygdala volume, these studies report mean estimates only, do not assess protective factors that may buffer effects of violence, and generally do not posit a cellular mechanism.[28,79] Future studies investigating for buffering effects in both human and animals may provide additional insight into operative neurodevelopmental mechanisms.

Notably, we found no evidence suggesting differences in amygdala volume mediated the relationship between violence exposure and behavior problems. While the amygdala is central to threat response, other brain regions are involved in this process as well. Because our analyses do not account for these other brain regions, our mediation models may be insufficient to even partially explain neurobiological mechanisms linking violence exposure and behavior problems. Moreover, it is also possible that violence exposure in our population-based sample was less acute than in prior case-control studies, which may have made it more difficult to detect whether differences in amygdala volume explain behavior.

We also found no evidence suggesting the effect of violence differed between boys and girls or among strata of parental socioeconomic status (SES). The latter finding is particularly surprising given the pervasive impact of SES on nearly all domains of health.[100] However, socioeconomic inequality (e.g., by income, wealth, etc.) is substantially less acute in the Netherlands than in the United States, where most other relevant studies were based, and the social gradient may be less influential.[101–103] Social assistance programs in the Netherlands are

also more generous than those in the United States, which may further reduce health inequalities.[104] These disparate findings across samples warrant additional research on the social determinants of neurodevelopment and behavior in children.

Our study has multiple implications for research investigating developmental impacts of childhood violence exposure. First, for studies assessing behavioral outcomes, our results further contextualize the association between violence exposure and behavior problems by quantifying an increase in both overall behavior problems and in specific behavior problem subtypes, e.g., aggressive behaviors. Second, our results suggest a healthy family environment may buffer some but not all of these effects. For example, healthy family functioning did not modify the effects of violence exposure on internalizing behaviors. These results are also significant because most related research investigates parenting practices (e.g., maternal support) as buffering elements of the family environment, while our measure of family functioning is directed more at a family's ability to support each other and solve problems. Finally, and perhaps most importantly, for studies investigating neurobiological outcomes, our results suggest childhood violence exposure may cause opposite effects depending on the family environmental context within which the violence occurs. Future research should either recruit participants from a broader range of social and economic backgrounds or assess carefully whether selection into the study sample limits the generalizability of the study's results.

Our study has some limitations. It is effectively cross-sectional, and we did not have data to assess within-person change in amygdala volume after violence exposure. Reverse causation could account for our results if children with higher levels of behavior problems were more likely to induce exposure to violence. Mothers reported their child's exposure to violence retrospectively, which may have led to recall bias. We did not account for exposure frequency,

severity, or timing, which may be important to neurodevelopment. Despite extensive confounding adjustment using prospectively collected data, residual and unmeasured confounding could affect our results. Finally, differential attrition from the study by salient sociodemographic characteristics may have induced selection bias, though our use of inverse probability weights to account for differential loss to follow up should partially mitigate this concern.

Our study also has several strengths. Our study design leveraged interview-based exposure data and questionnaire-based family functioning data from mothers, objective outcome data from MRI scans, and behavioral outcome data from fathers. This significantly lowers the risk of common method bias that may arise when the same reporter provides both exposure and outcome data.[75] Even where we used data from the same reporter, e.g., from mothers for violence exposure and family functioning, the data were collected using different methods (interviews, questionnaires) at different times, thereby reducing the risk that answers to one instrument influenced the mother's responses to the other. Compared with most case-control neuroimaging studies, our sample was large, population-based, and relatively diverse in childhood experiences. Finally, our relatively large sample also increased our statistical power relative to smaller studies, an important consideration when modeling interaction.

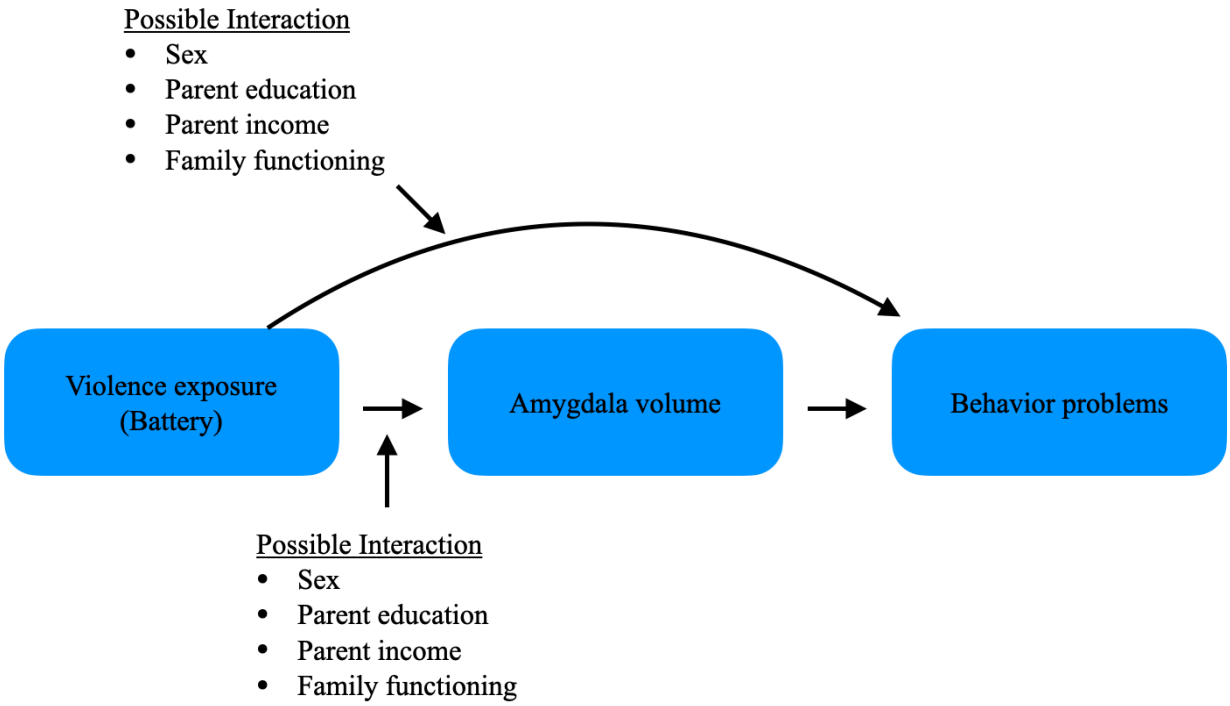
Conclusion

In a large, population-based neuroimaging birth cohort, higher levels of family functioning—a measure of the general childhood family environment—substantially buffered the association between higher levels of childhood violence exposure and higher levels of preadolescent behavior problems. Family functioning also altered the association between

violence exposure and amygdala volume. Our results suggest a healthy family environment may blunt deleterious neurodevelopmental consequences of childhood violence exposure. Future neuroimaging studies should consider effect modification by social environmental variables, and they should emphasize recruiting participants from a broad spectrum of childhood backgrounds and life experiences.

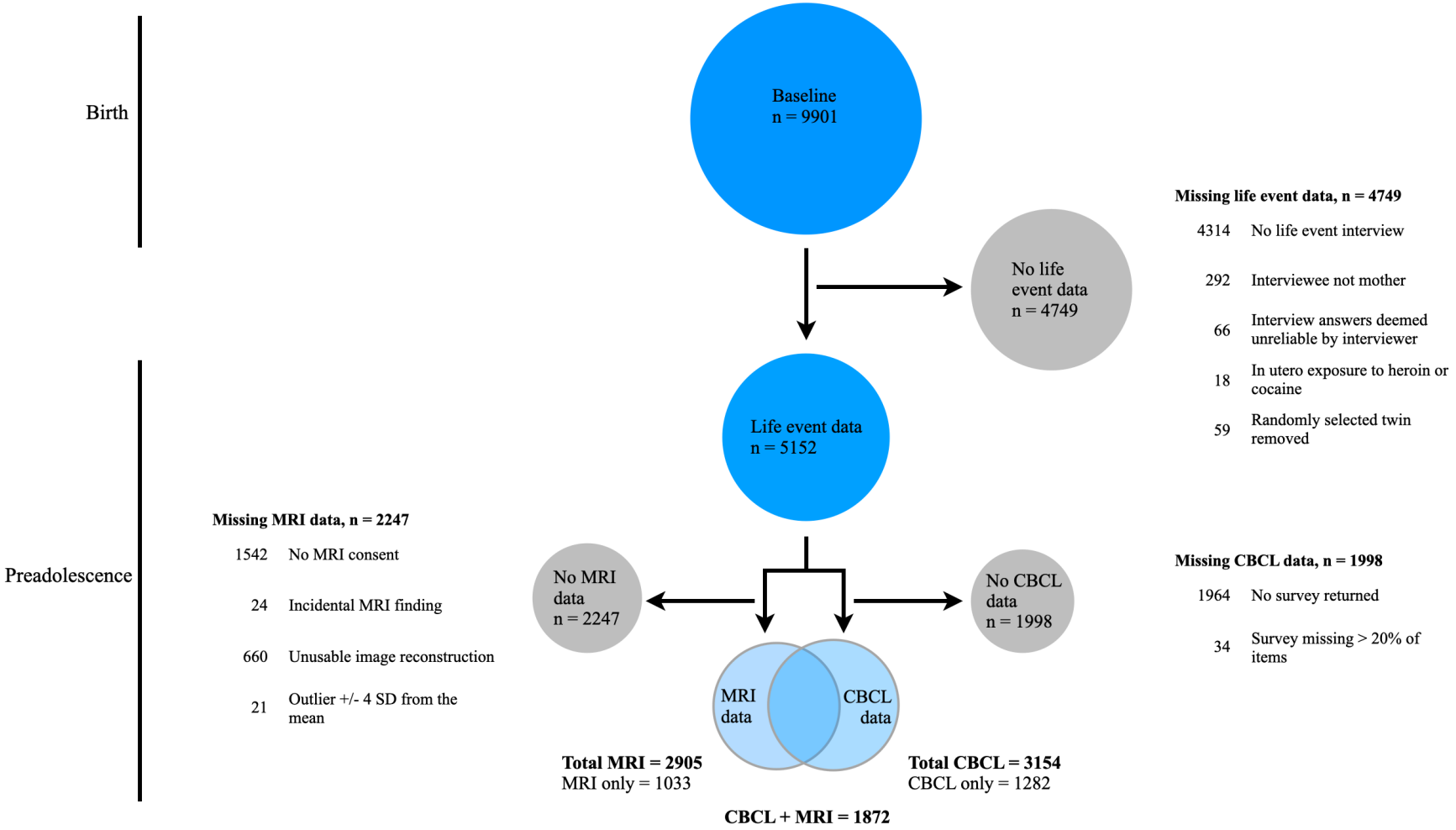
Appendix of Supplemental Information

SECTION 3.1: RELATIONSHIPS OF INTEREST IN THIS STUDY.



SECTION 3.2: SAMPLE CHARACTERISTICS.

3.2.1. Sample attrition from baseline.



3.2.2. Demographic characteristics of included versus excluded participants in analysis subsamples.

	Total MRI Sample n = 2905		Total CBCL Sample n = 3154		CBCL + MRI Sample n = 1872	
	Included	Excluded	Included	Excluded	Included	Excluded
Female	51%	49%	51%	48%	50%	49%
European (non-Turkish)	70%	58%	81%	51%	81%	57%
High parent income	50%	32%	61%	26%	61%	32%
High parent education	57%	38%	67%	33%	68%	38%

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

3.2.3. Additional sample characteristics.

Supplement Section 3.2.3. Distribution of family functioning scores and outcome measures by participant sociodemographic characteristics in primary analytic samples.

	Amygdala Volume Sample, n = 2,905				Behavior Problem Sample, n = 3,154			
	Family		Amygdala		Family		Total Behavior	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Total Analytic Sample	2.5	0.5	0.0	1.0	2.5	0.4	17.3	15.2
Child biological sex								
Female	2.5	0.5	-0.4	0.9	2.5	0.4	15.6	13.6
Male	2.5	0.5	0.4	1.0	2.5	0.4	19.0	16.5
Child ethnicity / country of origin								
Dutch / Other European	2.5	0.4	0.1	1.0	2.5	0.4	17.4	15.0
Turkish	2.5	0.5	0.0	1.0	2.4	0.5	13.9	14.4
Moroccan	2.3	0.5	-0.2	1.0	2.3	0.4	12.9	13.5
Surinamese	2.4	0.5	-0.4	1.0	2.4	0.4	16.2	13.6
Other	2.3	0.5	-0.1	1.0	2.4	0.5	19.7	18.0
Highest Household Education								
Less than high school equivalent	2.3	0.5	-0.2	1.1	2.3	0.5	19.1	18.8
High school or intermediate vocational train	2.4	0.5	-0.1	1.0	2.4	0.4	18.4	16.4
Adv. vocational training, bachelor's, or high	2.5	0.4	0.1	1.0	2.5	0.4	16.8	14.4
Household Income								
€2200 / month or less	2.4	0.5	-0.1	1.0	2.4	0.5	18.4	16.8
More than €2200 / month	2.5	0.4	0.1	1.0	2.5	0.4	16.5	14.0

1. This table is based on observed values for each characteristic and does not account for missing data.

2. Amygdala volumes in this table are unadjusted for head size.

3.2.4. Prevalence of additional measures of experiences of violence.

Supplement Section 3.2.4: Odds of threatening life events, mean harsh parenting scores, and mean corporal punishment scores by actual violence exposure status.

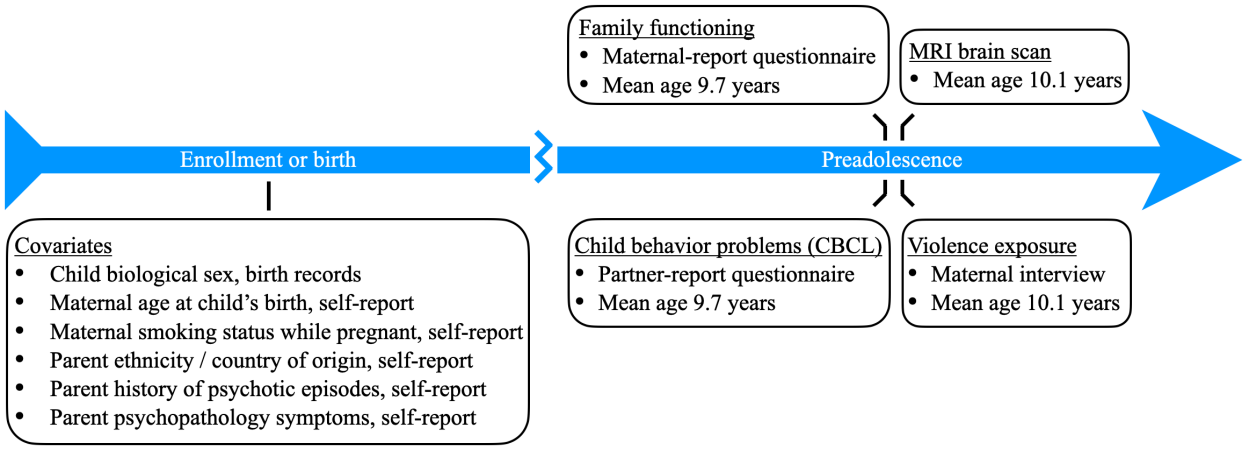
	Actual Violence		Odds
	Yes	No	Ratio
Family Conflict			
Yes	44	177	3.5
No	331	4598	
Non-Family Conflict			
Yes	116	351	5.6
No	259	4423	

	$\bar{x}$ (s)	$\bar{x}$ (s)	*p*
Maternal Harsh Parenting	0.51 (0.04)	0.49 (0.01)	0.51
Paternal Harsh Parenting	0.40 (0.03)	0.38 (0.01)	0.61
Corporal Punishment	0.31 (0.03)	0.28 (0.01)	0.40

1. These values are based on observed data only for each variable and do not account for missing data.
2. p-values are for two sample t-test assuming equal variances.

SECTION 3.3: MEASURES.

3.3.1. Timing of data collection.



3.3.2. Additional measures of experiences of violence.

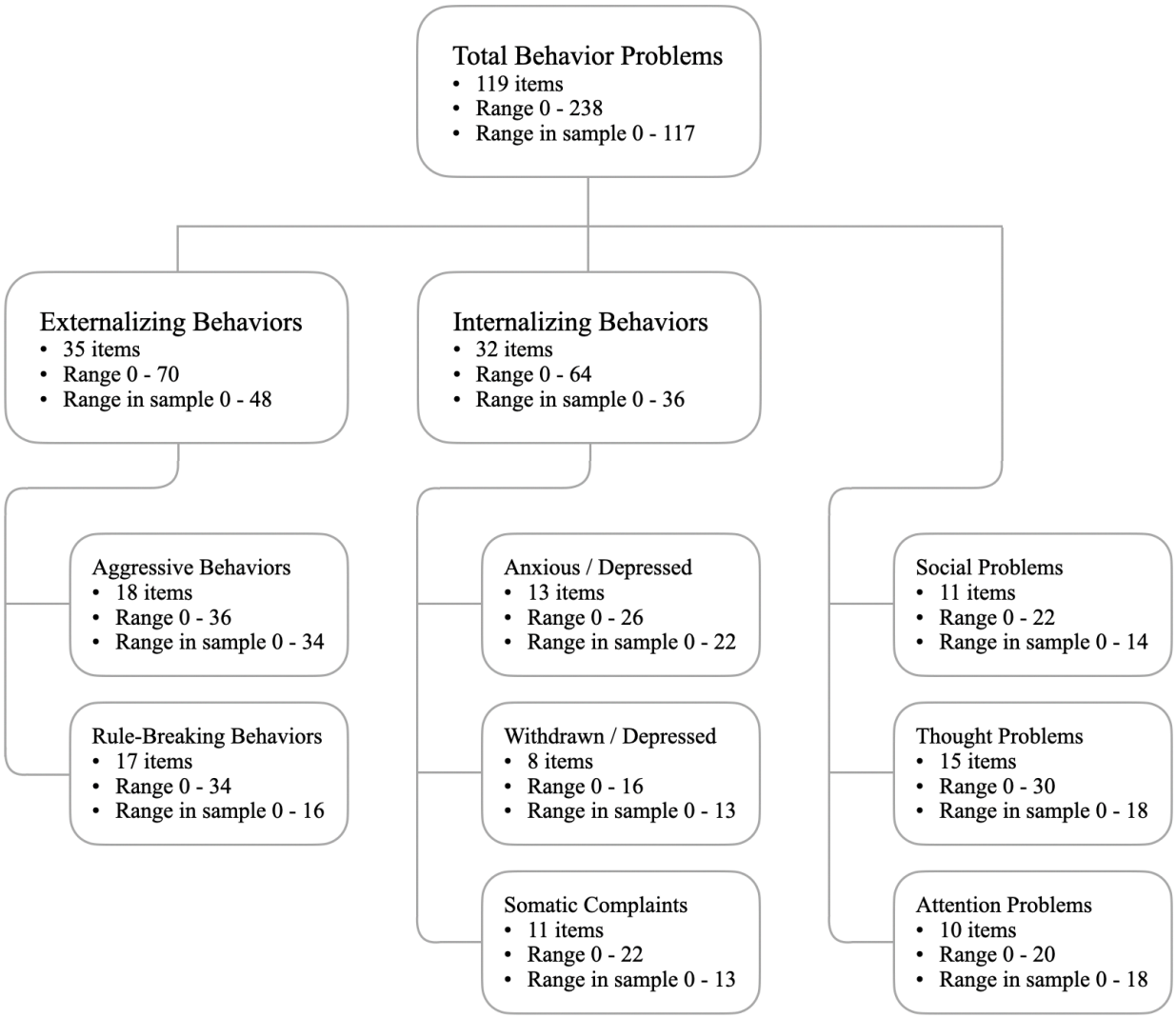
Stressful life events interview. At the same in-person study center visit when children were mean age 10.1 years (range 8.6 – 12.0), i.e., when mothers reported their child's violence exposure, study staff also asked mothers about their child's lifetime exposure (yes, no) to (1) ongoing conflict with a family member and (2) ongoing conflict with a non-family member. Specifically, the English translation of the questions asked in Dutch were (1) "Does your child have ongoing conflict with someone from the family (or has your child ever had this)?" and (2) "Does your child have ongoing conflict with someone else (or has your child ever had this)?"

Harsh Parenting Tactics. Generation R measured harsh parenting tactics used by mothers and partners separately with the Parent-Child Conflict Tactics Scale (CTS) via postal questionnaire.[70] When their children were aged 3.1 years (range 2.8 to 4.3), 4,908 mothers completed the CTS, 3,481 of whom Generation R staff later completed the stressful life events interview when their children were aged 10.1 years. Separately, 4,059 partners did the same, with the mothers of 2,976 of these children later completing the stressful life events interview.

Parents reported how often they engaged in 6 types of verbally or physically abusive disciplinary tactics during the preceding two weeks on a six-point frequency scale ranging from “never” to “more than 4 times”. English translations of representative items administered in Dutch included “I shook my child,” “I threatened to give a slap but I didn’t do it,” and “I called my child stupid or lazy or something like that.” We constructed a continuous sum score ranging from 0 to 30 to quantify overall harsh parenting exposure for each child participant.

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”.[72,73] 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.

3.3.3 Achenbach Child Behavior Checklist 6-18 structure.



Note: We did not independently test social problems, thought problems, and attention problems in this study because they are not included in the broader externalizing or internalizing subscales. We also did not test the subcomponents of the internalizing behavior subscale (i.e., anxious / depressed, withdrawn / depressed, somatic complaints) because no potential moderator modified the association between violence exposure and internalizing behaviors.

SECTION 3.4: STATISTICAL ANALYSIS METHODS.

3.4.1. Imputation models for missing data.

We imputed missing covariate data, missing CBCL data for participants with less than 20% missingness, and family functioning data. The proportion of missing data for most covariates was low (~ 3.0% for family functioning items, < 1.0% for most other variables), with the exception of household income (17.3%), partner educational attainment (26.1%), maternal psychopathology symptoms (20.9%), and partner psychopathology symptoms (28.7%). We used the 'mi impute chained' package in Stata 16.0/MP to conduct multiple imputation by chain 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 25 iterations to ensure convergence to a stationary posterior distribution. We created 50 imputed datasets and combined resulting estimates using Rubin's Rules.[59]

3.4.2. Inverse probability of attrition weights.

We defined participants lost to follow up as those enrolled at baseline but excluded from our analysis samples for any reason. We calculated a unique set of IPWs for each analysis sample. 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 16.0/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.[59] 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.

3.4.3. Mediation using bootstrapped linear regression models.

We conducted our mediation analyses consistent with a method for causal mediation analysis proposed by Valeri and VanderWeele (2013).[89] This method is implemented with the PARAMED package in Stata. However, because the PARAMED package cannot accommodate inverse probability of attrition weights, and because we did not hypothesize exposure-mediator interaction (which can be modeled using the PARAMED package), we used a series bootstrapped regression models within each of our 50 imputed datasets to estimate total, direct, and indirect effects along with standard errors of these estimates. We then combined these estimates using Rubin's Rules.[59]

Specifically, within each imputation, we first drew a bootstrapped sample. Next, we modeled the association between violence exposure and total behavior problems in a fully adjusted, weighted OLS regression model using the same set of covariates used throughout this study. Weights were inverse probability of attrition weights calculated previously. This produced an estimate of the total effect of violence exposure on behavior problems (i.e., $\beta_{\text{total effect / violence exposure}}$).

Next, we added a covariate for amygdala volume to the fully adjusted, weighted model used above. This model produced a new point estimate of the association between violence exposure and total behavior problems after accounting for differences in amygdala volume (i.e., $\beta_{\text{direct effect / violence exposure}}$). This is produced an estimate of the direct effect. Finally, we subtracted the direct effect estimate from the total effect estimate to produce an estimate of the indirect

effect (i.e., $\beta_{\text{total effect}} - \beta_{\text{direct effect}} = \beta_{\text{indirect effect}}$). We repeated this process on 1,000 bootstrapped samples within each imputation, which produced mean point estimates and standard errors across all bootstrapped samples within each of our 50 imputed datasets. Finally, we combined results from each imputed dataset using Rubin's Rules.

SECTION 3.5: ADDITIONAL TABLES AND RESULTS.

3.5.1: Total behavior problems, minimally versus fully adjusted models.

Supplement Section 3.5.1. Comparison of minimally adjusted and fully adjusted main effects and interaction estimates of the associations between maternal-report childhood actual violence exposure and partner-reported total behavior problems.

	Total Behavior Problems					
	Minimally adjusted models			Fully adjusted models		
	β	95% CI	*p*	β	95% CI	*p*
Main effect (no interaction)						
Violence exposure	14.50	(8.21, 20.82)	< 0.001	9.71	(3.86, 15.55)	< 0.01
Interaction models						
Sex						
Violence exposure	10.42	(1.55, 19.28)	0.02	4.58	(-1.22, 10.37)	0.12
Violence exposure * Boy	5.54	(-6.29, 17.36)	0.36	6.92	(-2.53, 16.38)	0.15
Parent Income						
Violence exposure	17.85	(8.50, 27.20)	< 0.001	11.50	(2.06, 20.94)	0.02
Violence exposure * Higher income	-9.32	(-22.22, 3.57)	0.16	-4.29	(-16.51, 7.93)	0.49
Parent Education						
Violence exposure	16.15	(6.04, 26.26)	0.00	10.80	(1.61, 19.98)	0.02
Violence exposure * Parent education	-5.43	(-22.52, 11.66)	0.53	-2.68	(-16.19, 10.84)	0.70
Family Functioning (continuous)						
Violence exposure	48.62	(16.39, 80.84)	< 0.01	39.27	(12.63, 65.91)	< 0.01
Violence exposure * Functioning	-15.42	(-29.01, -1.83)	0.03	-13.14	(-23.80, -2.49)	0.02
Family Functioning (binary)						
Violence exposure	28.30	(13.70, 42.90)	< 0.001	20.03	(7.65, 32.41)	< 0.01
Violence exposure * High functioning	-18.31	(-34.32, -2.30)	0.03	-13.44	(-27.50, 0.62)	0.06

1. Minimally adjusted models include covariates for child age at outcome assessment, sex, and ethnicity / country of origin.
2. Fully adjusted models include covariates for child age at outcome assessment, sex, ethnicity, and in utero smoking exposure; parental highest household education, household income, history of psychosis, and psychopathology symptoms; and maternal age at child's birth.
3. All estimates are weighted to reflect differential attrition from the baseline sample to the analysis sample; unweighted n for total behavior problems = 3,154.
4. The continous family functioning measure has range 0-3; the binary functioning measure is < or ≥ 2.0.
5. Total behavior problems are measured by the partner-reported CBCL total score; range in sample 0 - 117.

3.5.2. Amygdala volume, minimally versus fully adjusted models.

Supplement Section 3.5.2. Comparison of minimally adjusted and fully adjusted main effects and interaction estimates of the associations between maternal-report childhood actual violence exposure and preadolescent amygdala volume.

	Amygdala Volume					
	Minimally adjusted models			Fully adjusted models		
	β	95% CI	*p*	β	95% CI	*p*
Main effect (no interaction)						
Violence exposure	-0.21	(-0.38, -0.05)	0.01	-0.20	(-0.37, -0.03)	0.02
Interaction models						
Sex						
Violence exposure	-0.36	(-0.67, -0.06)	0.02	-0.33	(-0.64, -0.02)	0.04
Violence exposure * Boy	0.22	(-0.14, 0.57)	0.24	0.19	(-0.17, 0.55)	0.30
Parent Income						
Violence exposure	-0.11	(-0.35, 0.13)	0.36	-0.12	(-0.37, 0.12)	0.32
Violence exposure * Higher income	-0.19	(-0.59, 0.21)	0.36	-0.17	(-0.58, 0.23)	0.41
Parent Education						
Violence exposure	-0.09	(-0.35, 0.17)	0.49	-0.09	(-0.36, 0.17)	0.49
Violence exposure * Parent education	-0.23	(-0.61, 0.16)	0.25	-0.20	(-0.59, 0.19)	0.31
Family Functioning (continuous)						
Violence exposure	1.01	(0.27, 1.75)	0.01	1.00	(0.23, 1.76)	0.01
Violence exposure * Functioning	-0.52	(-0.84, -0.21)	< 0.01	-0.51	(-0.84, -0.19)	< 0.01
Family Functioning (binary)						
Violence exposure	0.24	(-0.03, 0.51)	0.08	0.24	(-0.03, 0.53)	0.08
Violence exposure * High functioning	-0.56	(-0.88, -0.24)	< 0.01	-0.54	(-0.87, -0.21)	< 0.01

1. Minimally adjusted models include covariates for child age at outcome assessment, sex, and ethnicity / country of origin.
2. Fully adjusted models include covariates for child age at outcome assessment, sex, ethnicity, and in utero smoking exposure; parental highest household education, household income, history of psychosis, and psychopathology symptoms; and maternal age at child's birth.
3. All estimates are weighted to reflect differential attrition from the baseline sample to the analysis sample; unweighted n for total behavior problems = 2,905.
4. The continous family functioning measure has range 0-3; the binary functioning measure is < 2.0 / >= 2.0.
5. Amygdala volume is assessed as the standardized bilateral mean for each participant.

3.5.3: Results from models testing sex, parental education, and parental income as moderators of the relationship between violence exposure and behavior problem subtypes.

Supplement Section 3.5.3. Additional interaction estimates by sex, parental income, and parental education of the associations between childhood violence exposure and behavior problem subtypes.

	Externalizing Behaviors									Internalizing Behaviors		
	Overall Externalizing Score			Aggressive Behavior Score			Rule-breaking Behavior Score			Overall Internalizing Score		
	β	95% CI	*p*	β	95% CI	*p*	β	95% CI	*p*	β	95% CI	*p*
Interaction models												
Sex												
Violence exposure	0.98	(-1.06, 3.02)	0.35	0.78	(-0.67, 2.22)	0.29	0.20	(-0.50, 0.90)	0.57	2.05	(-0.50, 4.59)	0.12
Violence exposure * Boy	2.59	(-0.73, 5.92)	0.13	2.44	(-0.12, 5.00)	0.06	0.16	(-0.82, 1.13)	0.75	0.57	(-2.60, 3.74)	0.72
Parent Income												
Violence exposure	3.58	(0.15, 7.01)	0.04	3.39	(0.65, 6.12)	0.02	0.19	(-0.69, 1.07)	0.67	2.73	(0.25, 5.22)	0.03
Violence exposure * Higher income	-1.62	(-6.12, 2.89)	0.48	-1.90	(-5.51, 1.71)	0.30	0.28	(-0.83, 1.39)	0.62	-0.64	(-3.78, 2.50)	0.69
Parent Education (binary)												
Violence exposure	2.95	(-0.43, 6.34)	0.09	2.85	(0.09, 5.60)	0.04	0.11	(-0.74, 0.96)	0.81	2.87	(0.48, 5.26)	0.02
Violence exposure * High education	-0.21	(-5.06, 4.64)	0.93	-0.68	(-4.65, 3.28)	0.74	0.47	(-0.74, 1.68)	0.45	-0.97	(-4.51, 2.57)	0.59

1. All models are fully adjusted and include covariates for child age at outcome assessment, sex, ethnicity, and in utero smoking exposure; parental highest household education, household income, history of psychosis, and psychopathology symptoms; and maternal age at child's birth.

2. All estimates are weighted to reflect differential attrition from the baseline sample to the analysis sample; unweighted n for total behavior problems = 3,154.

3. All outcome measures are continuous with the following ranges in this sample: internalizing behaviors 0 - 36; externalizing behaviors 0 - 48; aggressive behaviors 0 - 34; rule-breaking behaviors 0 - 16.

4. Because our exposure did not vary across all strata of our three-category parental education variable used elsewhere in this study, analyses for this table used a binary parental education variable (< or ≥ advanced vocational training, bachelor's degree, or higher).

3.5.4. Mediation model results.

Supplement Section 3.5.4: Mediation of the association between actual violence exposure and total behavior problems by amygdala volume in pre-adolescence.

	n	β	95% CI	*p*
Full sample				
Total Effect	1872	9.30	(2.50, 16.10)	0.01
Direct Effect		9.24	(2.59, 16.23)	0.01
Indirect Effect		0.05	(-0.29, 0.40)	0.77
Stratified by family functioning scor	n	β	95% CI	*p*
Functioning Score >= 2.0	1659			
Total Effect		7.60	(0.62, 14.59)	0.03
Direct Effect		7.22	(0.42, 14.03)	0.04
Indirect Effect		0.38	(-0.26, 1.02)	0.25
Functioning Score < 2.0	213			
Total Effect		14.82	(-3.94, 33.57)	0.12
Direct Effect		14.01	(-4.33, 32.35)	0.14
Indirect Effect		0.81	(-2.28, 3.90)	0.62

These fully-adjusted models include covariates for child age, sex, ethnicity, and in utero exposure to smoking, and parental highest household education, household income, history of psychosis, psychopathology symptoms, and maternal age at birth.

Conclusion

Findings from research in this dissertation are broadly consistent with the conceptual model on which it is based. These studies provide evidence supporting the central premise that both positive and negative childhood social exposures have independent and interacting effects on brain structure and behavior. Specifically, Chapter 1 of this dissertation found that one positive aspect of the early-life social environment—namely, prenatal healthy family functioning—was associated with greater global white matter microstructural integrity in preadolescence. This finding provides the first evidence linking a positive prenatal exposure to white matter microstructure. It suggests a healthy prenatal social environment may confer lasting neurodevelopmental benefits on children, thereby underscoring the importance of the family environment even before children are born.

Next, Chapter 2 of this dissertation found that one negative aspect of the early-life social environment—namely, childhood exposure to actual violence—was associated with decreases in both global cortical and subcortical volumes and in volumes of specific corticolimbic regions of interest, including the amygdala. Notably, Chapter 2's analyses found no evidence that exposure to mere threatened violence in childhood was associated, on average, with preadolescent global brain morphology. Together, these findings suggest both that negative social exposures in childhood may affect brain structure, and that even qualitatively similar (but not identical) negative social exposures may have different neurodevelopmental consequences.

Finally, in Chapter 3, our results suggest healthy childhood family functioning alters the relationship between actual violence exposure and both amygdala volume and behavior problems in preadolescence. Moreover, it appears that among children exposed to actual violence, those who also had healthy family functioning reported fewer behavior problems. These findings

suggest aspects of positive and negative social environments interact with each other in complex ways to affect childhood brain and behavioral development.

Together, these studies contribute to research investigating brain-based mechanisms linking childhood risk and protective factors to the development of childhood behavior problems. Exploring these biological mechanisms is important to public health because doing so sheds light on whether and how specific social exposures are most likely to affect health and development. In turn, public health researchers and practitioners may better direct scarce resources both within the research community and toward the development of more effective public health programs and policies aimed at reducing the burden of childhood behavior problems and increasing overall child wellbeing.

These studies also contribute to two emerging domains within epidemiology. The first is positive epidemiology, which explores both how positive protective factors may influence health and how positive health and wellbeing may manifest beyond the mere absence of disease.[105] Specifically, the family functioning score used in Chapters 1 and 3 measures a family's capacity to solve problems and accept each other as they are (i.e., positive exposures)—in essence, it is a composite representing aspects of family harmony and social support. This is in contrast to other measures of the family environment that often assess only the presence or absence of family conflict (i.e., a negative exposure). While the substantial weight of epidemiologic and neurodevelopmental research focuses on negative exposures and negative outcomes, new research in positive epidemiology suggests positive social, psychosocial, and environmental exposures may affect health and wellbeing as much as negative exposures. This dissertation—and particularly Chapter 1—adds to this growing body of research.

Findings from these studies also contribute to a second emerging domain of epidemiology; namely, population neuroscience. This cross-disciplinary field leverages tools and data from epidemiology, biostatistics, psychology, and neuroscience to answer a broad array of questions about how genetic and environmental factors influence brain phenotype and function, and about how brain phenotype may either mediate or predict a range of mental and physical health outcomes. Population neuroscience is made possible by the advent of large-scale neuroimaging studies. The past decade has seen a dramatic increase in the number of research cohorts collecting MRI data from large numbers of participants (i.e., more than 1,000). These cohorts are generally (though not exclusively) conceived and followed by neuroscientists, psychologists, and applied physicists, who bring a deep understanding of the brain, its function, and how to measure it using various techniques, including magnetic resonance imaging. However, these cohorts are substantially larger than samples previously used in neuroscience studies, which often entailed fewer than, say, 300 participants, and they often collect more phenotypic data over more time as well. As such, studies using data from these large neuroimaging cohorts benefit from methods and tools developed or advanced in epidemiology and biostatistics, which have long traditions of analyzing observational data in large samples, but which lack familiarity with brain imaging techniques. By merging expertise from neuroscience, psychology, epidemiology, and biostatistics, population neuroscience offers promising new opportunities to explore determinants of population health and disease.

This cross-disciplinary approach has several specific strengths. The first relates to measurement. Brain-based measures—essentially, neural biomarkers—provide objective biological outcomes that can be used to complement subjective measures of behaviors, psychological wellbeing, and psychopathology often used in psychiatric epidemiology.

Moreover, brain-based measures are particularly well-suited to study how social experiences are biologically embodied because one of the brain's core functions is to receive external stimuli, process it, and develop a response. In this way, it is extremely sensitive to experiential input, changing its physical structure and function in response to it. Moreover, specific regions of the brain are responsible for controlling the body's stress response mechanisms. Stress is a major pathway thought to link social adversity to health, making the brain a natural target for investigating stress-based hypotheses of biological embodiment. As such, brain phenotypes in children may provide an early marker of healthy development, or they may be used to predict future health challenges. In the latter case, researchers may seek effective interventions leveraging the developing brain's substantial plasticity to mitigate the risk of health challenges later in life.

A second strength of population neuroscience is that it advances the study of biological mechanisms linking social, psychosocial, and environmental exposures to health. Studying mechanisms may prove particularly helpful in explaining heterogeneous outcomes associated with a certain exposure. For example, new research has identified four distinct biotypes of depression based on unique patterns of dysfunctional brain connectivity.[106] These biotypes could not be differentiated on the basis of clinical features, yet they predicted which participants would benefit from certain therapies. By extension, future research may seek to identify distinct biotypes associated with childhood adversity, which in turn may identify how and why some children facing adversity are resilient and others are not. Similarly, researchers may explore whether population neuroscience can reveal optimism biotypes with the aim of understanding with greater specificity how and for whom optimism protects against disease. Thus, population neuroscience may identify rich new areas of research.

A third important advantage of population neuroscience is as a tool for science communication. While this advantage does not relate directly to scientific investigation, it is still squarely within public health practice. Viewing health and disease through a brain-based lens may provide a powerful frame that can be used to galvanize support for public health programs and policies more effectively than less provocative (though perhaps better established) types of research.

Despite these advantages, the field of population neuroscience must surmount several challenges if it is to realize its potential. Chiefly, causal links between brain phenotype and behavior are still poorly understood. This is evident in Chapter 3, which notes that both larger and smaller amygdala volumes have been associated with increased behavior problems in children. Much more research is required to understand how brain phenotypes translate to behavior and health. Another challenge to population neuroscience includes the expense and challenge of collecting brain scan data, which can take between 30 and 60 minutes per participant depending on scan protocols. Current research rates for MRI scan time on the type of scanner most commonly used in research hover between $300 and $600 per hour, which makes scanning large cohorts prohibitively expensive for all but the most well-resourced studies. Processing scans is also computationally expensive, adding to the financial burden. Moreover, many participants are unable to receive MRI scans, while others are unable to lie still in an MRI scanner for long periods of time. This may induce substantial selection bias. While researchers are likely to solve some of these challenges, others are likely to remain for the foreseeable future.

This dissertation's research can be extended in multiple ways. Most immediately, findings from Chapter 3, in which healthy family functioning alters the relationship between violence exposure and amygdala volume, warrant additional scrutiny. Future research may

attempt a form of replication of these findings by assessing whether similar interaction occurs with related measures of both physically threatening experiences (e.g., neighborhood violence exposure) and positive family environmental exposures (e.g., healthy parental sensitivity). Separately, researchers may explore whether the same exposures investigated in this dissertation are also associated with other measures of brain phenotype, including measures of functional connectivity and brain network topology. This would contribute a deeper understanding of the mechanisms this dissertation seeks to explore.

Future research should also investigate the neurodevelopmental effects other important social exposures. The family environment offers a compelling target for investigation because (1) the public health burden of child mental health problems remains high and continues to increase; (2) prior research has demonstrated the importance of the family environment to healthy child development; and (3) the family environment can entail both negative (e.g., family conflict, child maltreatment) and positive (e.g., family cohesion, parental support) experiences. Given that the substantial weight of prior evidence investigates negative exposures, the effects of positive social exposures both within the family and outside of it are largely unexplored, offering fertile ground for research into adolescent wellbeing and flourishing.

More broadly, population neuroscience offers opportunity to explore social and environmental determinants of brain health among both children and adults of all ages. Beyond social and psychosocial exposures, researchers may use large neuroimaging cohorts to investigate how environmental toxins, including lead and heavy metals, affect brain development and cognition as well as the onset of cognitive decline. Brain health effects of climate change, including exposure to severe weather events (heat waves, natural disasters, etc.), can also be studied using population-based neuroimaging datasets for the first time. Thus, with productive

collaboration across disciplines, population neuroscience offers many new, significant, and innovative opportunities to advance both basic health research and the public's health.

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