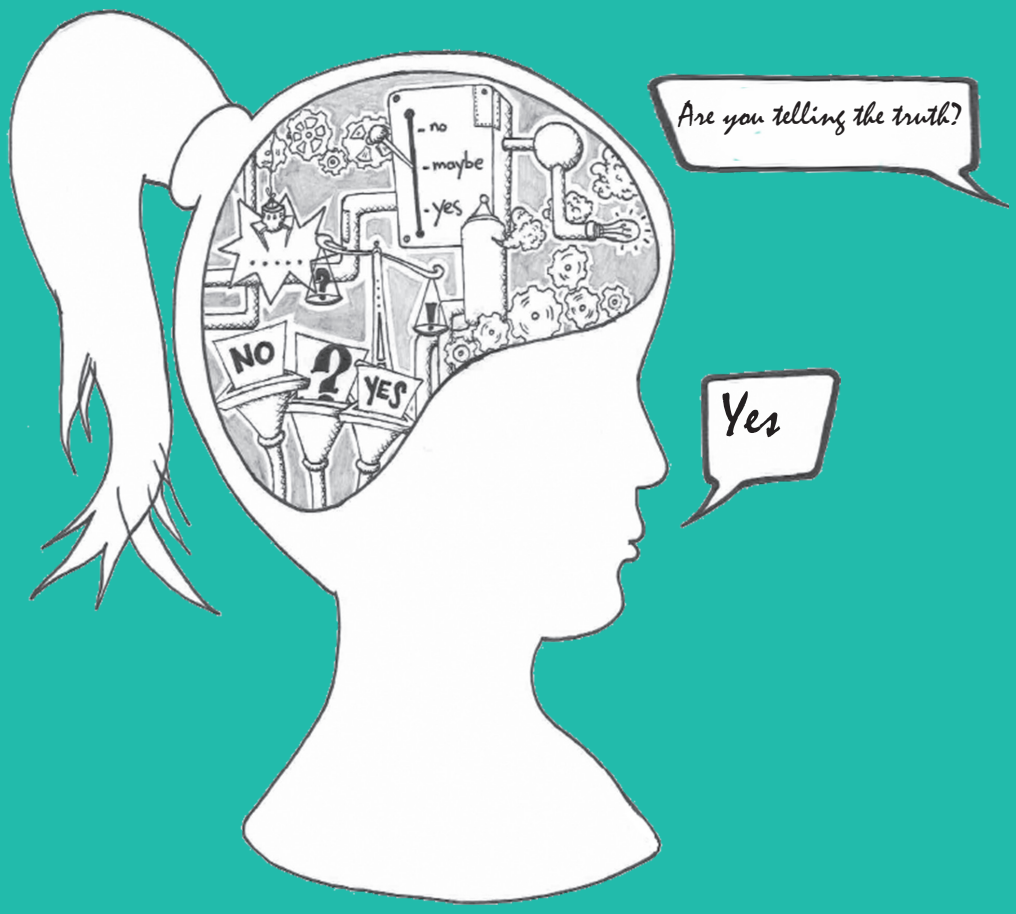
Neurobiological Correlates of Externalizing and Prosocial Behavior in School-Age Children

A study on truths and lies



Sandra Thijssen

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A study on truths and lies

Neurobiologische correlaten van externaliserend gedrag en prosociaal gedrag in schoolgaande kinderen

Een onderzoek naar waarheden en onwaarheden

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Chapter 1

General introduction

Externalizing behaviors, such as aggressive behavior and persistent dishonesty, tend to begin very early in life and persist across different developmental stages (Loeber, 1982). Overall, child-onset externalizing problems are predictive of later psychopathic personality traits, mental-health problems, substance dependence, and drug-related and violent crime (Moffitt, Caspi, Harrington, & Milne, 2002). While early externalizing problems are related to later antisocial behavior, several factors may decrease the negative outcomes associated with externalizing behavior problems observed in adolescence or adulthood. For example, an intervention focusing on prosocial behavior has been shown to reduce externalizing problems in children (Vliek, Overbeek, & de Castro, 2014). Moreover, factors outside of the child, such as quality of parenting, play an important role in the development of later antisocial behavior (Taylor, Manganello, Lee, & Rice, 2010), and intervention studies focusing on sensitive parenting have shown to be effective in decreasing externalizing behavior (Bakermans-Kranenburg, Van IJzendoorn, Pijlman, Mesman, & Juffer, 2008; Moss et al., 2011; Van Zeijl et al., 2006). Examining the neurobiological correlates of externalizing behaviors, prosocial behavior and parenting quality may provide critical information for our understanding of the etiology of antisocial behavior. Moreover, as brain development is plastic, understanding the early neurobiology of externalizing behaviors and prosocial behavior, as well as understanding the effect of parenting quality on brain development, may facilitate the development of early intervention programs. This thesis aims to describe our work on the neurobiological correlates of externalizing behavior as well as prosocial behavior in six-to ten- year old children.

BRAIN MORPHOLOGY OF AGGRESSION AND PROSOCIAL BEHAVIOR

There is accumulating evidence that both aggressive behavior and prosocial behavior may be associated with specific neurobiological factors (Boes, Tranel, Anderson, & Nopoulos, 2008; Ducharme et al., 2011; Siever, 2008; Sterzer & Stadler, 2009). Imaging studies of adult and adolescents have associated antisocial behavior with structure and function of the amygdala, hippocampus, anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) (Kiehl, 2006; Siever, 2008). In children, precursors of antisocial behavior have been studied in the context of two disorders characterized by high levels of aggression: conduct disorder (CD) and oppositional defiant disorder (ODD), which have been related to functional and structural abnormalities in the dorsolateral prefrontal cortex (DLPFC), OFC, ACC, amygdala, and hippocampus (Fairchild et al., 2013; Fairchild et al., 2011; Gavita, Capris, Bolno, & David, 2012; Huebner et al., 2008; Matthys, Vanderschuren, & Schutter, 2013). Current knowledge on the neurobiology of prosocial behavior predominantly stems from functional magnetic resonance imaging (MRI) studies in adults, that suggest a role for the DLPFC and the medial PFC in prosocial behavior (Mathur, Harada, Lipke, & Chiao, 2010; Rameson, Morelli,

& Lieberman, 2012; Waytz, Zaki, & Mitchell, 2012). As the brain undergoes considerable changes during childhood and adolescence, it is currently unclear whether these findings also apply to children. Moreover, differences in brain functioning and structure related to adult behavior may be affected by the behavior itself (reversed causality) or environmental influences. Studying the neurobiological correlates of behavior in children increases the chance of identifying structures that are involved in the etiology of the behavior (Sterzer & Stadler, 2009).

SITUATIONAL MORALITY

In contrast to a trait-like interpretation of behavior, externalizing and prosocial behavior may also be the product of the demand-characteristics of the situation. Several widely known social-psychological experiments, such as the Milgram experiments or the Stanford prison experiment, have demonstrated that situational characteristics may drive healthy adults to commit inhumane acts (Haney, Banks, & Zimbardo, 1973; Milgram, 1963). Situational influences have also been demonstrated in children. For example, the Milgram experiment has been repeated in children showing similar results (Shanab & Yahya, 1977). At the same time, differences in situational characteristics may also prompt individuals to show more appropriate behavior. Studies show that children as young as five years of age share more and steal less when they are being observed by a peer (Engelmann, Herrmann, & Tomasello, 2012), and even the presence of an imaginary but invisible person prevents children from cheating (Piazza, Bering, & Ingram, 2011). Situational pressures can override genetic, dispositional or personality differences in determining choices (Van IJzendoorn, Bakermans-Kranenburg, Pannebakker, & Out, 2010). Therefore, it is important to determine to what extent externalizing behaviors are persistent across situations. Moreover, it may be important to examine the effects of demand-characteristics of the situation on functional brain correlates of externalizing behaviors.

DISHONEST BEHAVIOR

Lie-telling, one of the main examples of externalizing behavior, may be specifically dependent upon situational characteristics. While some lying may be normative, problems arise when lying becomes persistent across situations and damages the interests of others (Talwar & Crossman, 2011). Although a number of studies have examined the typical development of lie-telling in children, less is known about children who lie persistently across situations. As persistent dishonest behavior may pose problems for children themselves as well as their environment, examining correlates of persistent lie-telling may provide important insights into child externalizing problems.

Functional neuroimaging studies in adults suggest that the prefrontal cortex and ACC play an important role in dishonest behavior (Abe, 2011; Christ, Essen, Watson, Brubaker, & McDermott, 2009; Greene & Paxton, 2009; Ito et al., 2011; Langleben et al., 2002; Lee, Lee, Raine, & Chan, 2010; Spence et al., 2001). These regions have been implicated in cognitive processes related to deception, such as working memory, inhibitory control, and error detection (Abe, 2011). However, not much is known about neural activation of deception in children, nor about neural activation underlying typical versus persistent lie-telling.

AIMS

The aim of the studies described in this thesis is to examine the neurobiological correlates of externalizing as well as prosocial behavior in six-to ten- year old children. More specifically, chapter 2 and 3 present our findings on the brain morphology of aggressive and prosocial behavior, respectively. In chapter 4, we present our findings on the correlates of typical and persistent dishonest behavior. In chapter 5, we examined the effect of situational characteristics on the neural correlates of lie-telling. Finally, in chapter 6 we examined the association between parental sensitivity during early childhood and brain morphology later in childhood. Chapter 7 provides a general discussion of the research findings, important considerations and practical implications.

SETTING

The studies described in this thesis are embedded in the Generation R study, a prospective cohort from fetal life onwards in Rotterdam, the Netherlands (Jaddoe et al., 2012; Tiemeier et al., 2012). In brief, 9778 pregnant women living in Rotterdam (delivery dates between April 2002 and January 2006) were enrolled through midwives and obstetricians. During the prenatal phase, at preschool age and at age 6 years, regular extensive assessments (e.g. questionnaires and observations) have been carried out in children and their parents. At the data collection wave at age 6 years 8.305 children were still participating.

The structural and functional neuroimaging data collection as described in the current thesis took place from September of 2009 until August 2013 (White et al., 2013). Structural MRI data has been collected in 1070 children. Inclusion was based on specific criteria for recruitment, such as specific prenatal exposures and behavioral phenotypes. Exclusion criteria included contraindications for the MRI procedure (i.e., pacemaker, ferrous metal implants), severe motor or sensory disorders (deafness or blindness), neurological disorders (i.e., seizures or tuberous sclerosis), and moderate to severe head injuries with loss of consciousness, and claustrophobia. For the structural MRI studies described here, children

included based on behavioral phenotypes other than externalizing and prosocial behavior were excluded from analyses. For the functional neuroimaging studies described in the present thesis, we recruited groups of highly aggressive children, highly prosocial children, and control children (Wildeboer et al., 2015).

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Chapter 2

Brain morphology of childhood aggressive behavior: a multi-informant study in school-age children

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ABSTRACT

Objective: Few studies have focused on the neuroanatomy of aggressive behavior in children younger than 10 years. Here, we explored the neuroanatomical correlates of aggression in a population-based sample of six-to-nine year old children using a multiple-informant approach.

Methods: Magnetic Resonance (MR) scans were acquired from 566 children of the Generation R study who participated in the Berkeley Puppet Interview and whose parents had completed the Child Behavior Checklist. Linear regression analyses were used to examine associations between aggression and amygdala and hippocampal volume. We performed surface-based analyses to study the association between aggression and cortical thickness, surface area and gyrification.

Results: Aggressive behavior was associated with smaller amygdala ($p<.05$), but not hippocampal volume. Aggression was associated with a thinner cortex in the left precentral cortex ($p<.01$) and in a cluster including the right inferior parietal, supramarginal, and postcentral cortex ($p< .001$). Gender moderated the association between aggression and cortical thickness in the right medial posterior cortex ($p=.001$), and the right prefrontal cortex ($p<.001$). Aggression was associated with decreased gyrification in a large cluster including the right precentral, postcentral, frontal, and parietal cortex ($p=.01$). Moreover, aggression was associated with decreased gyrification in the right occipital and parietal cortex ($p=.02$).

Conclusions: We found novel evidence that childhood aggressive behavior is related to decreased amygdala volume, decreased sensorimotor cortical thickness and decreased global right hemisphere gyrification. Aggression is related to cortical thickness in regions associated with the default mode network, with negative associations in boys and positive associations in girls.

INTRODUCTION

There is accumulating evidence that aggressive behavior may be associated with specific neurobiological factors (Boes, Tranel, Anderson, & Nopoulos, 2008; Ducharme et al., 2011; Siever, 2008; Sterzer & Stadler, 2009). Imaging studies of adult and adolescent aggression have repeatedly reported associations with the amygdala, hippocampus, anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) (Siever, 2008). However, differences in brain functioning and structure related to adult aggression may be affected by the behavior itself (reversed causality) and/or environmental influences (e.g. lead exposure). Studying the neurobiological correlates of aggression in children increases the chance of identifying structures that are involved in the etiology of aggression (Sterzer & Stadler, 2009). Compared to adult and adolescent aggression, aggressive behavior in childhood has been relatively understudied. Imaging studies that have focused on childhood aggression have often examined the neurobiology of aggression in clinical samples. Examining the neuroanatomy of normal variation in aggressive behavior may provide information critical to our understanding of normal human behavior as well as psychopathology (Ducharme et al., 2011). Moreover, while there is general consensus that child behavior requires assessment from multiple informants (Kraemer et al., 2003), most neuroimaging studies on childhood aggression rely only on parent-reported aggression data (Ameis et al., 2014; Ducharme et al., 2011; Visser et al., 2014). In the present study, we explored the neuroanatomical correlates of normal variation in aggressive behavior in a large population-based sample of six-to-nine year old children using a multiple-informant approach.

Individuals expressing high levels of aggressive behavior may be viewed as having "a lower threshold for activation of motoric aggressive responses to external stimuli without adequate reflection or regard for the aversive consequences of the behavior" (p.340) (Siever, 2008). This may point to an imbalance between limbic drives, such as the amygdala, and prefrontal control mechanisms. Functional Magnetic Resonance Imaging (fMRI) research has shown that aggressive adults display increased amygdala reactivity and decreased OFC activation in response to angry faces (Coccaro, McCloskey, Fitzgerald, & Phan, 2007). In highly aggressive individuals, diminished connectivity between the amygdala and the OFC has been observed (Coccaro et al., 2007). Moreover, the structure of the amygdala, OFC, ACC, and the dorsolateral prefrontal cortex (DLPFC) are also related to aggression (Schiffer et al., 2011; Yang & Raine, 2009).

In children, aggressive behavior has been studied in the context of two disorders characterized by high levels of aggression: conduct disorder (CD) and oppositional defiant disorder (ODD). Both disorders have been related to functional and structural abnormalities in the DLPFC, OFC, ACC, amygdala, and hippocampus (Fairchild et al., 2013; Fairchild et al., 2011; Gavita, Capris, Bolno, & David, 2012; Huebner et al., 2008; Matthys, Vanderschuren, & Schutter, 2013). Previous studies of children and adolescents have reported negative

correlations between aggressive behavior and ACC and OFC thickness in boys and girls (Ameis et al., 2014; Boes et al., 2008; Ducharme et al., 2011; Walhovd, Tamnes, Ostby, Due-Tonnessen, & Fjell, 2012), and increased hippocampus volume in girls only (Visser et al., 2014). Gender differences in both aggression (Alink et al., 2006; Borsa, Damasio, Bandeira, & Gremigni, 2013) and neurobiological development (Mutlu et al., 2013; Raznahan et al., 2010) have been amply documented. There is also evidence that the neuroanatomical correlates underlying childhood aggression may show gender related differences (Ducharme et al., 2011; Fairchild et al., 2013; Visser et al., 2014).

Previous normative neuroimaging studies on childhood aggression in non-clinical samples have mostly included samples covering a wide age range (± 10 years) and/or a mean age above 10 years (Ameis et al., 2014; Ducharme et al., 2011; Visser et al., 2014; Walhovd et al., 2012). However, in childhood and young adolescence the brain is developing rapidly. In general, cortical gray matter development is characterized by a nonlinear growth curve, with gray matter volume reaching its peak around puberty (Giedd et al., 1999). Regions associated with more primary functions develop earlier compared to regions involved in more complex tasks (e.g. prefrontal and temporal cortex) (Gogtay et al., 2004). Since the cortical areas that are most often related to aggressive behavior reach their cortical peak after age 10, focusing on the brain of young children may provide information on the developmental neurobiology of childhood aggression that might otherwise go unnoticed. Moreover, many studies on the neuroanatomical correlates of aggression have focused on cortical gray matter volume. However, cortical volume is a function of cortical thickness and cortical surface area. Cortical thickness and surface area can be viewed as separate endophenotypes, reflecting the underlying genetic influence on brain development (Rakic, 1995). Cortical gyrification (cortical folding) is an important property of the cortex that helps to increase cortical surface area within a confined space (Reillo, Romero, Garcia-Cabezas, & Borrell, 2011). Focusing on cortical thickness and cortical area separately and additionally examining cortical gyrification provides complementary information on the brain morphology of childhood aggression.

The present study assessed the association between the normal variation in aggressive behavior and cortical thickness, surface area, gyrification and amygdala and hippocampal volume in a large population-based sample of six-to-nine year old children using combined parent-reported and child-reported measures of aggression. We hypothesized that aggression would be related to reduced amygdala and hippocampal volume. Although previous studies strongly imply an association between the prefrontal cortex and aggression, this association may not yet be fully present in six-to-nine year olds. Based on prior studies showing gender differences in aggressive behavior as well as cortical development, we also expected gender-related differences in the association between brain morphology and aggression.

METHODS

Participants

Participants were recruited from the Generation R study, a population-based prospective cohort from early fetal life onwards in Rotterdam (Jaddoe et al., 2012; Tiemeier et al., 2012). From 799 eligible six-to-nine year old children with structural imaging data, 117 (15%) children were missing parent and/or child reported aggression scores. From the remaining 682 children, 90 (11%) had poor quality T1 data. Seven twin pairs were excluded from the analyses and from seven sibling pairs, one child was randomly excluded. As our aggression measures were suitable for children younger than 8 years, 5 children were excluded who fell above this cut-off. Consequently, cortical surface analyses were performed on 566 children (285 boys). This study was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. All parents provided informed consent.

Measures

Aggressive behavior – parent report

The primary caregiver completed the Child Behavior Checklist 1½ – 5 (CBCL 1½ – 5) (Achenbach & Rescorla, 2000) when the children were on average 6 years old. We chose to use the CBCL 1½ – 5 years to enable the use of the same measure of parent-reported aggressive behavior for the entire sample. Although the average age of the sample was 6 years at time of the aggression assessment, the majority of the children (60%) was still 5 years of age. CBCL can be scored on eight scales, including the Aggressive Behavior scale that was used in the present study (M=5.38, SD=4.68). This scale consists of 19 items scored on a 3-point Likert scale (α=.87). To approach normality, Aggressive Behavior scores were square root transformed (Tabachnick & Fidell, 2007).

Aggressive behavior – child report

Self-reported aggressive behavior measures were obtained using the Berkeley Puppet Interview (BPI) (Ablow, Measelle, & Assessment, 2003) when the children were on average 6 years old. Details on BPI data collection in Generation R have been described elsewhere (Ringoot et al., 2013). Briefly, two identical hand puppets named "Iggy" and "Ziggy" presented the children with opposing statements. The child was asked to indicate which statement described him or her best. Videotaped responses were scored on a 7-point Likert scale (intraclass correlation for intercoder reliability .96-.98), with higher scores representing more problems.

We used the broadband Externalizing scale -including the Oppositional Defiant, Overt Hostility and Conduct Problems scales- as an indicator of aggressive behavior. We choose to use this scale (α=.77, M=51.69, SD=10.33) as it was most comparable to the CBCL Aggressive Behavior scale. For example, CBCL Aggressive Behavior items such as "Screams"

or "Disobedient" reflect Oppositional Defiant items in the BPI ("When I don't get my own way, I don't yell at my teacher/mom or dad" "I do what my teacher asks me to do"). Items such as "Hits others", "Attacks people" or "Destroys others' things" reflect items in the BPI Overt Hostility and Conduct Problems scales ("I don't hit my mom or dad/ my teacher or other grown-ups", "I don't fight with other kids", "I don't break other peoples' things when I'm mad at them"). To approach normality, the Externalizing scale was inverse transformed (Tabachnick & Fidell, 2007) and then standardized to increase interpretability.

Combining parent and child reported data

In order to obtain a multiple-informant aggressive behavior score, we aggregated scores reported by the parent and the child. Parent and child scores showed a correlation of r=.12. Generally, agreement between parent and child informants tends not to exceed 0.20 (Kraemer et al., 2003). Low levels of agreement between informants suggest that childhood functioning is best conceptualized as the separate and combined influences of children's actual characteristics, the context in which children are observed, the perspectives (or biases) of the informants, and error of measurement. Kraemer at al. (2003) therefore suggest that the choice of informants should be based on consideration of the contexts and perspectives that influence the characteristic under investigation. Weaknesses of one informant should be compensated by strengths of another. While parents observe their child only in the home environment, the child itself can report on his or her behavior in all contexts. Furthermore, children provide information on how they perceive themselves, while parents provide an other-report. As recommended by Kraemer et al. (2003), we performed an unrotated principle component analysis on the items of the two scales. The first component provides a multiple-informant measure of aggression that is relatively free of informant bias, whereas the second component is thought to reflect rater differences. We also created a multiple-informant aggression scores by calculating the mean of the standardized untransformed parent and child scores. Since the correlation between the averaged multiple-informant aggression score and the multiple-informant component coefficient was extremely high (r=.93), we decided to use the averaged aggression score for further analyses. The associations between the multiple-informant score and the parent and child reported scores each amounted to r=.75. The multiple-informant aggression score was square root transformed to approach normality.

Covariates

Information on gender, gestational age, and date of birth was obtained from midwives and hospital registries. Ethnicity, maternal education level and smoking during pregnancy were assessed through questionnaires. Handedness was measured using a modified version of the Edinburgh Handedness Inventory (Oldfield, 1971). Intelligence (IQ) was estimated from the Mosaics and Categories subtest of the Snijders-Oomen Non-Verbal Intelligence

Test-Revised (Tellegen, Winkel, Wijnberg-Williams, & Laros, 2005). The prosocial scale of the Strengths and Difficulties Questionnaire (SDQ) was used as a measure of prosocial behavior (Goodman, 1997; Paap et al., 2013). Attention problems and internalizing problems were measured using the CBCL (Achenbach & Rescorla, 2000). Parental psychopathology was measured using the anxiety and depression scales of the Brief Symptom Inventory (Derogatis & Melisaratos, 1983). Models were adjusted for covariates that generated a change in predictor effect estimates of 5% or more.

Structural Magnetic Resonance Imaging

A description of the neuroimaging component of the Generation R study has been described elsewhere (White et al., 2013). Children were 6 to 9 years of age at the time of the MRI assessment. On average, time between aggressive behavior assessment and the MRI was 1.76 years. Prior to the MRI, the children were first familiarized with a mock scanning session. MRI scanning was performed on a GE Discovery MR 750 3 T scanner (General Electric, Milwaukee, MI, USA). T1-weighted inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence was obtained using an 8 channel head coil with the following parameters: TR=10.3 ms, TE=4.2 ms, TI=350 ms, NEX=1, flip angle=16°, readout bandwidth=20.8 kHz, matrix 256x256, imaging acceleration factor 2, and an isotropic resolution of 0.9x0.9x0.9 mm^3.

Image Processing

Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite 5.1 (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications (Reuter, Schmansky, Rosas, & Fischl, 2012). Briefly, this process included the removal of non-brain tissue, automated Talairach transformation into standard space, and segmentation of the subcortical white and gray matter volumetric structures (including the amygdala and hippocampus), intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction, and surface deformation.

Once the cortical models were complete, the images underwent surface inflation (Fischl, Sereno, & Dale, 1999), registration to a spherical atlas (Fischl, Sereno, Tootell, & Dale, 1999), and the parcellation of the cerebral cortex into units based on gyral and sulcal structure (Desikan et al., 2006). Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/cerebrospinal fluid boundary at each vertex on the tessellated surface (Fischl & Dale, 2000). The surface based map was smoothed with a 10 mm full-width half-maximum (FWHM) Gaussian kernel prior to the surface based analyses. Several studies using Freesurfer in typically and atypically developing school-aged children are available (El Marroun et al., 2014; Juuhl-Langseth et al., 2012).

To assess the local gyrification index (LGI) we used the method of Schaer et al. (2008), that is implemented in FreeSurfer. This approach provides an estimation of the local gyrification index, taking into account the three-dimensional cortical surface. Identification of the pial and white matter surfaces against an additional surface that tightly wraps the pial surface are used to estimate the degree of cortical folding at a 25 mm spherical vertex-based region. This method has been validated and used in several studies focusing on childhood and adolescent psychopathology (Schaer et al., 2013; Wallace et al., 2014). The surface based LGI maps were smoothed prior to the analyses using a 5 mm FWHM Gaussian kernel, consistent with comparable studies (Wallace et al., 2014). Gyrification data was available on 557 children.

Image quality

All unprocessed T1 images were visually inspected at the scan site. Raters were instructed to assess movement and scanner artifacts on a 6-point scale (unusable, poor, fair, good, very good, excellent). After processing through the FreeSurfer pipeline, overall segmentation quality of the images was inspected on a 7-point scale (not constructed, poor, fair, fairly good, good, very good, excellent). Unprocessed images rated as unusable or poor were excluded from the analyses (*n*=31), as were images that could not be processed by FreeSurfer and images with a poor segmentation quality (*n*=59). We additionally rated amygdala and hippocampal segmentation quality as usable or unusable. Scans with unusable hippocampal or amygdala segmentation quality (*n*=66) were excluded from the amygdala and hippocampal analyses only.

Statistical analyses

Missing values on the covariates (1 – 23%) were imputed using the predictive mean matching method. Results of 10 imputed datasets were pooled to obtain an overall outcome.

Hierarchical linear regression analyses were used to examine the association between aggressive behavior and amygdala and hippocampal volume. Amygdala and hippocampal volume were residualized for total brain volume (TBV). In the first block, gender, age, IQ and aggression were entered. In the second step, the covariates were entered (gestational age, ethnicity, prosocial behavior, maternal education, and maternal smoking during pregnancy), and in the third step the gender-by-aggression interaction term.

We performed vertex-wise exploratory analyses of the association between aggression and cortical thickness, cortical surface area and cortical gyrification across the entire cortex. Furthermore, we examined whether gender moderated the association between cortical thickness, surface area or gyrification and aggression. For this purpose, FreeSurfer's QDEC was used (www.surfer.nmr.mgh.harvard.edu). Regions for which cortical thickness, surface area or gyrification was significantly associated with aggressive behavior as well as regions in which gender moderated this association were determined using general linear

models (GLMs) with age, gender, and IQ as covariates. For use in QDEC, missing values on intelligence were mean imputed. To correct for the effect of multiple comparisons, a cluster based Monte Carlo simulation was performed using 10,000 iterations and $p \leq .05$.

Surface-based Regions Of Interest (ROIs) were manually created for brain areas that were significantly related to aggression and utilized to extract the mean cortical thickness, surface area or gyrification within the specific ROI for every participant. ROIs were then residualized for TBV. Hierarchical linear regression analyses were used to examine the effect of confounding variables on the extracted ROI (gender, age, IQ and aggression in the first block, covariates in the second). Age-by-ROI and gender-by-age-by-ROI interaction effects were also tested. As these effects were not significant, they are not reported.

To confirm our primary findings, we bootstrapped the GLM analysis using 500 iterations of 200 participants (Efron & Tibshirani, 1993). For each voxel, *p-value*s were averaged to generate an overall result. Moreover, analyses were repeated for CBCL Aggressive Behavior and BPI Externalizing Behavior scores separately. These results are provided as Supplemental Material (Supplementary Text 2.1, Supplementary Table 2.1 and Supplementary Figure 2.1 and 2.2).

RESULTS

Demographic characteristics of the sample can be found in Table 2.1. For all assessments, boys and girls were comparable in age (*ps*>.15). Boys had higher aggression scores than girls ($F(1,564)=11.64$, $p=.001$, $d=0.28$ for CBCL, $F(1,564)=47.62$, $p<.001$, $d=0.57$ for BPI, and $F(1,570)=45.93$, $p<.001$, $d=0.57$ for the multiple-informant aggression score). Age at assessment was not related to aggressive behavior. There were negative correlations between IQ and aggressive behavior using the multiple-informant aggression score, $r=-.12$, $p<.01$, the parent-reported CBCL $r=-.10$, $p=.03$, and the child-reported BPI $r=-.08$, $p=.06$. Prosocial behavior was negatively related to aggressive behavior for the multiple-informant score, $r=-.21$, $p<.001$, and for the parent-reported CBCL $r=-.24$, $p<.001$, but not for the child-reported BPI $r=-.05$, $p=.30$.

Association between aggressive behavior and amygdala and hippocampal volume

Table 2.2 shows the associations between aggressive behavior and amygdala and hippocampal volume. Aggression was associated with a smaller amygdala volume, $\beta=-.12$, $p<.05$ for total amygdala volume, and $\beta=-.11$, $p=.03$ and $\beta=-.08$, $p=.11$, for right and left amygdala volume respectively. Aggressive behavior was not associated with hippocampal volume, $\beta=-.02$, $p=.76$. Scatterplots of the association between aggressive behavior and amygdala and hippocampal volume are provided in Supplementary Figure 2.3.

Table 2.1 | Demographic Characteristics

***N*=566**	***M*(*SD*)/*n*(%)**		***M*(*SD*)/*n*(%)**
Boys	285(50.4%)	Maternal Education	
Ethnicity		Primary or lower	11(1.9%)
Dutch	433(76.5%)	Secondary	213(37.6%)
Other-Western	33(5.8%)	Higher	333(58.8%)
Non-Western	100(17.7%)	Maternal smoking during pregnancy	
Gestational age	40.06(1.63)	Never	437(77.2%)
IQ	104.08(14.02)	Quit when pregnancy	34(6.0%)
Right-handed	517(90.5%)	known	
SDQ Prosocial Behavior	13.33(1.79)	Continued	91(16.1%)
		Parental psychopathology	
CBCL Aggressive Behavior	5.38(4.68)	Depression	1.34(2.53)
Age CBCL	6.03(0.41)	Anxiety	1.47(2.31)
CBCL filled out by			
Mother	521(91.8%)		
Father	37(6.5%)		
Both	6(1.1%)		
Other	1(0.2%)		
BPI Externalizing	51.69(10.33)		
Age BPI	6.11(0.39)		
Age MRI	7.87(0.97)		

Table 2.2 | Associations Between Aggressive Behavior and Amygdala and Hippocampal Volume (*n*=500)

		Total			Left			Right		
		B(95% CI)	**β**	***part r***	**B(95% CI)**	**β**	***part r***	**B(95% CI)**	**β**	***part r***
Amygdala	Baseline adjusted[1]	-93.14 (-201.12; 14.84)	-.08	-.06	-34.62 (-91.25; 22.01)	-.06	-.05	-54.38 (-119.99; 11.24)	-.08	-.07
	Adjusted[2]	-136.11 (-259.03; -13.18)	-.12*	-.10	-52.16 (-116.39; 12.07)	-.08	-.07	-81.09 (-155.95;-6.23)	-.11*	-.10
Hippo-campus	Baseline adjusted[1]	39.76 (-124.68; 204.20)	.02	.02	49.42 (-40.68; 139.51)	.05	.05	-11.73 (-103.03; 79.57)	-.02	-.01
	Adjusted[2]	-29.47 (-216.84; 157.90)	-.02	-.01	5.50 (-96.63; 107.64)	.01	.01	-35.43 (-139.83; 68.96)	-.03	-.03

Note. * $p<.05$. Amygdala and hippocampus volume were corrected by TBV.

[1] Adjusted for gender and age

[2] Adjusted for gender, age, IQ, gestational age, ethnicity, handedness, attention problems, internalizing problems, prosocial behavior, image quality, maternal education, parental psychopathology, maternal smoking during pregnancy

Association between aggressive behavior and cortical thickness

Aggressive behavior was associated with reduced cortical thickness in a cluster including the left precentral cortex (1150 mm^2, max vertex X=-52.5, Y=-6.6, Z=38.5, p=.005) and a cluster including the right inferior parietal, supramarginal, and postcentral cortex (2139 mm^2, max vertex X=42.9, Y=-27.6, Z=37.6, p<.001, Figure 2.1a). Gender moderated the association between aggression and cortical thickness in a cluster including the right precuneus, isthmus of the cingulate cortex and lingual cortex (1344 mm^2, max vertex X=8.5, Y=-53.1, Z= 0.4, p=.001), as well as in a cluster covering the right middle and superior frontal cortex (2067 mm^2, max vertex X=34.4, Y=49.8, Z=7.7, p<.001, Figure 2.1b).

To examine the effect of possible confounding variables on the association between the ROIs and aggression, hierarchical linear regression models were used. Greater aggressive behavior was associated with reduced cortical thickness of the left precentral ROI and the right postcentral ROI, β=-.14, p=.005 and β=-.25, p=.001 respectively (Table 2.3). For regions in which there was a significant gender-by-aggression interaction effect, analyses were repeated for boys and girls separately (Table 2.4). In girls, aggressive behavior was associated with a thicker cortex in the right precuneus ROI and the right frontal ROI, β=.16, p=.02 and β=.22 p<.001 respectively. In boys, aggression was not related to the right precuneus ROI, β=-.12, p=.07. In the right frontal ROI, we found a negative association between cortical thickness and aggressive behavior in boys, β=-.14, p=.04. To assure that the gender-by-aggression interaction effect was not caused by gender differences in mean levels of aggression, we repeated the analysis on a subset of 190 boys and 190 girls with equivalent aggression scores. Results were similar. To confirm our primary findings, we bootstrapped the GLM analysis using 500 iterations of 200 participants. Supplementary Figure 2.4 shows a cortical map of the averaged *p-value*s across the 500 bootstraps. Although cluster size may differ, all clusters were confirmed.

Table 2.3 | Associations Between Aggressive Behavior and Cortical Thickness (n=566)

		B(95% CI)	β	*part r*
L precentral ROI	Baseline adjusted[1]	-0.10(-0.15;-0.04)	-.15***	-.14
	Adjusted[2]	-0.09(-0.15;-0.03)	-.13**	-.11
R postcentral ROI	Baseline adjusted[1]	-0.11(-0.16;-0.07)	-.20***	-.19
	Adjusted[2]	-0.09(-0.14;-0.04)	-.15**	-.13

Note. ** p<.01*** p<.001. Cortical thickness was corrected by TBV.

[1] Adjusted for age, IQ and gender

[2] Adjusted for age, gender, IQ, ethnicity, attention problems, internalizing problems, prosocial behavior, image quality, maternal smoking during pregnancy

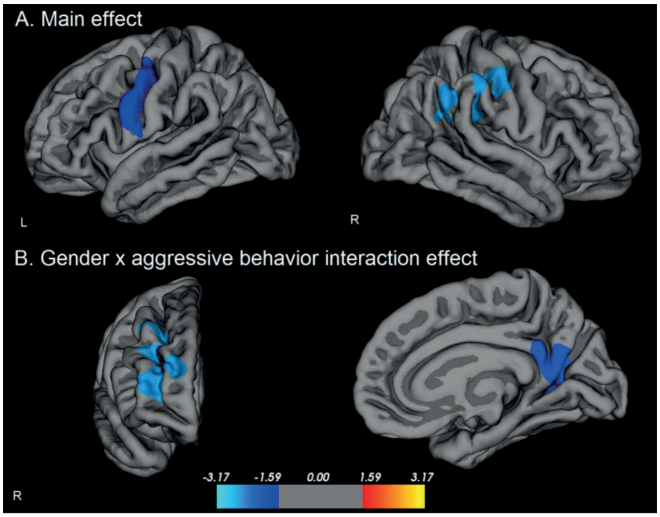

Figure 2.1 | Relation between cortical thickness and aggressive behavior. Sex, age and IQ were used as covariates (Monte Carlo corrected cluster-wise $p<.05$). A. Cortical thickness was negatively associated with aggression in a cluster covering the left precentral cortex and a cluster covering the right inferior parietal, supramarginal, and postcentral cortex (i.e. reduced cortical thickness was associated with more aggressive behavior). B. A moderating effect of gender was found for a cluster including the right middle frontal, and superior frontal cortex and for a cluster including the right precuneus, isthmus of the cingulate cortex and lingual cortex, with negative associations in boys and positive associations in girls. Colors represent –log10 *p-value*.

Association between aggressive behavior and cortical surface area

The surface-based whole brain analysis of the association between cortical surface area and aggressive behavior provided no significant results.

Association between aggressive behavior and gyrification

Figure 2.2 shows the association between aggressive behavior and gyrification. Aggressive behavior was associated with decreased gyrification in a cluster including the left precentral cortex, extending to the postcentral, paracentral, parietal, temporal, occipital, precuneus, and inferior frontal cortex (24314 mm^2, max vertex X=-36.8, Y=-18.3, Z=64.5, $p<.001$), as well as in a cluster including the left rostral middle frontal cortex (3010 mm^2, max vertex X=-22.1, Y=41.5, Z=24.1, $p=.001$). In the right hemisphere, aggressive behavior was associated with deceased gyrification in a cluster including the precentral cortex, extending anteriorly to the middle and superior frontal cortex, and posteriorly to postcentral, superior parietal, and supramarginal cortex (11807 mm^2, max vertex X=27.7, Y=-14.4, Z=60.2, $p=.001$). Moreover, we found a negative association between aggression and gyrification in a cluster including the postcentral, and insular cortex (6060 mm^2, max vertex X=62.1, Y=-9.6, Z=28.9, p=.001), as well as in a cluster including the lateral occipital and inferior parietal cortex

Table 2.4 | Gender Moderating Effects in Cortical Thickness ROIs (*n*=566)

		Aggression x gender			Boys			Girls		
		B(95% CI)	**β**	***part r***	**B(95% CI)**	**β**	***part r***	**B(95% CI)**	**β**	***part r***
R precuneus ROI	Baseline adjusted[1]	-0.29(-0.44;-0.14)	-.24***	-.15	-0.10(-0.20;-0.01)	-.13*	-.12	0.17(0.05;0.29)	.17**	.17
	Adjusted[2]	-0.27(-0.42;-0.12)	-.23***	-.15	-0.10 (-0.21;0.01)	-.12	-.10	0.17 (0.02;0.31)	.16*	.13
R frontal ROI	Baseline adjusted[1]	-0.21(0.27;-0.16)	-.25***	-.16	-0.09(-0.16;-0.01)	-.14*	-.14	0.13(0.05;0.21)	.19**	.19
	Adjusted[2]	-0.21(-0.31;-0.11)	-.25***	-.16	-0.09(-0.16;0.01)	-.14*	-.12	0.15(0.07;0.24)	.22***	.19

Note. * $p<.05$ ** $p<.01$*** $p<.001$. Cortical thickness was corrected by TBV.

[1] Adjusted for age, IQ (and gender)

[2] Adjusted for age, IQ, (gender), ethnicity, attention problems, internalizing problems, prosocial behavior, image quality, maternal smoking during pregnancy

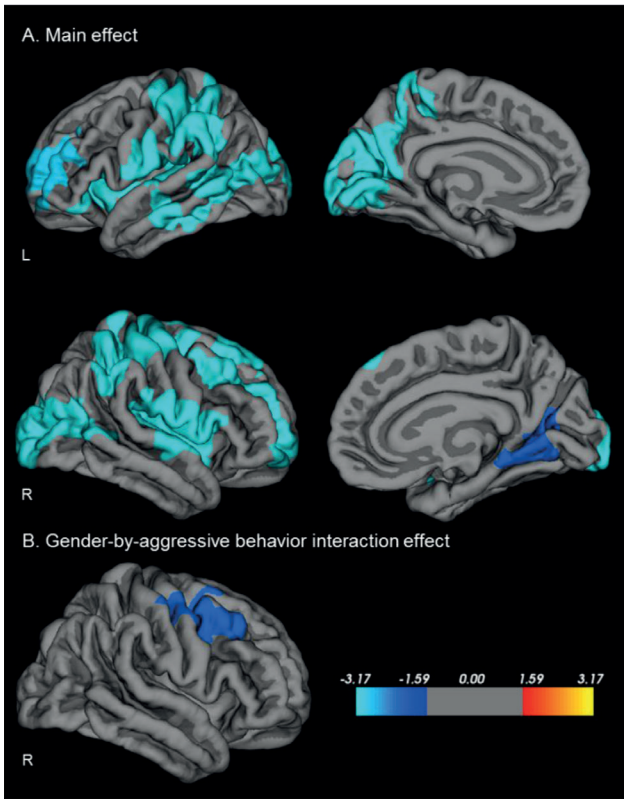

Figure 2.2 | Relation between cortical gyrification and aggressive behavior. Sex, age and IQ were used as covariates (Monte Carlo corrected cluster-wise $p<.05$). A. Cortical gyrification was negatively associated with aggression in a cluster covering the left precentral cortex, extending to the postcentral, paracentral, parietal, temporal, occipital, precuneus, and inferior frontal cortex as well as in a cluster including the left rostral middle frontal cortex (i.e.reduced gyrification was associated with more aggressive behavior). In the right hemisphere, aggressive behavior was associated with reduced gyrification in a cluster including the precentral cortex, extending anteriorly to the frontal cortex, and posteriorly to postcentral, and parietal cortex. The second right hemisphere cluster included the postcentral, and insular cortex. The third right hemisphere cluster included the lateral occipital and inferior parietal cortex, while the forth cluster included the lingual cortex, extending to the precuneus and cuneus. B. A moderating effect of gender was found for a cluster including the right middle frontal, and superior frontal cortex and for a cluster including the right right precentral, postcentral, frontal, and supramarignal cortex. In this region, greater aggressive behavior was associated with reduced gyrification in boys only. Colors represent –log10 *p-value*.

(5355 mm^2, max vertex X = 21.3, Y = -98.7, Z = 5.3, p = .001), and in a cluster including the lingual cortex, extending to the precuneus and cuneus (2568 mm^2, max vertex X=25.4, Y=-61.6, Z=0.7, p=.01). Gender moderated the association between aggressive behavior and gyrification in a cluster including the right precentral, postcentral, and caudal middle

frontal cortex (5762 mm^2, max vertex X=27.7, Y=-14.4, Z=60.2, p<.001). This cluster overlaps with the right hemisphere main effect precentral cluster.

Moreover, we found a negative association between aggression and gyrification in a cluster including the postcentral, and insular cortex (6060 mm^2, max vertex X=62.1, Y=-9.6, Z=28.9, *p*=.001), as well as in a cluster including the lateral occipital and inferior parietal cortex (5355 mm^2, max vertex X=21.3, Y=-98.7, Z=5.3, *p*=.001), and in a cluster including the lingual cortex, extending to the precuneus and cuneus (2568 mm^2, max vertex X=25.4, Y=-61.6, Z=0.7, *p*=.01). Gender moderated the association between aggressive behavior and gyrification in a cluster including the right precentral, postcentral, and caudal middle frontal cortex (5762 mm^2, max vertex X=27.7, Y=-14.4, Z=60.2, *p*<.001). This cluster overlaps with the right hemisphere main effect precentral cluster.

Results of the hierarchical regression analyses correcting for total brain volume and confounding variables can be found in Table 2.5. After correction for confounding variables, aggression was associated with decreased gyrification only in the right precentral ROI and right lateral occipital ROI, β=-.13, *p*=.01 and β=-.10, *p*=.02, respectively. As the right precentral ROI overlaps with the gender-by-aggression interaction ROI, we additionally tested a gender-by-aggression interaction term. This interaction term was not significant, β=-.11, *p*=.10. For the right gender-by-aggression interaction ROI analyses were repeated for boys and girls separately (Table 2.6). In this region, more aggressive behavior was associated with reduced gyrification in boys only, β=-.19, *p*=.006. To assure that the gender-by-aggression interaction effect was not caused by gender differences in mean levels of aggression, we repeated the analysis on a subset of 190 boys and 190 girls with equivalent aggression scores. Results were similar.

Table 2.5 | Associations Between Aggressive Behavior and Gyrification (*n*=557)

		B(95% CI)	**β**	***part r***
L precentral ROI	Baseline adjusted[1]	-0.04 (-0.07; -0.00)	-.09*	-.09
	Adjusted[2]	-0.03 (-0.07; 0.01)	-.06	-.05
L rostral middle frontal ROI	Baseline adjusted[1]	-0.04 (-0.07; 0.00)	-.09	-.08
	Adjusted[2]	-0.03 (-0.07; 0.01)	-.08	-.07
R precentral ROI	Baseline adjusted[1]	-0.04 (-0.08; -0.00)	-.09*	-.09
	Adjusted[2]	-0.06 (-0.10; -0.01)	-.13*	-.11
R postcentral ROI	Baseline adjusted[1]	-0.03 (-0.11; 0.05)	-.03	-.03
	Adjusted[2]	0.02 (-0.07; 0.11)	.02	.02
R lateral occipital ROI	Baseline adjusted[1]	-0.05 (-0.09; -0.01)	-.12**	-.11
	Adjusted[2]	-0.04 (-0.09; -0.00)	-.10*	-.08
R lingual ROI	Baseline adjusted[1]	-0.06 (-0.13; 0.01)	-.07	-.07
	Adjusted[2]	-0.04 (-0.11; 0.04)	-.05	-.04

Note. * *p*<.05 ** *p*<.01. Gyrification was corrected by TBV.

[1] Adjusted for age, IQ and gender

[2] Adjusted for age, IQ, gender, ethnicity, attention problems, internalizing problems, prosocial behavior, image quality, maternal smoking during pregnancy

Table 2.6 | Gender Moderating Effect on Gyrification of Right Precentral ROI (*n*=557)

	Aggression x gender			Boys			Girls		
	B(95% CI)	**β**	***part r***	**B(95% CI)**	**β**	***part r***	**B(95% CI)**	**β**	***part r***
Baseline adjusted[1]	-0.14 (-0.19; -0.08)	-.17*	-.11	-0.08 (-0.16; -0.01)	-.13*	-.13	0.04 (-0.03; 0.11)	0.07	.07
Adjusted[2]	-0.13 (-0.24; -0.03)	-.17*	-.11	-0.12 (-0.20; -0.03)	-.19**	-.17	0.04 (-0.04; 0.12)	0.07	.06

Note. * $p<.05$ ** $p<.01$. Gyrification was corrected by TBV.

[1] Adjusted for age, IQ (and gender)

[2] Adjusted for age, IQ, (gender), ethnicity, attention problems, internalizing problems, prosocial behavior, image quality, maternal smoking during pregnancy

To confirm our primary findings, we bootstrapped the GLM analysis using 500 iterations of 200 participants. Supplementary Figure 2.5 shows a cortical map of the averaged *p-values* across the 500 bootstraps. All clusters were confirmed.

DISCUSSION

The present study examined the neuroanatomical correlates of aggressive behavior in six-to-nine year old children using a multiple-informant approach. As hypothesized, childhood aggression was associated with smaller amygdala volume. Moreover, aggressive behavior was associated with decreased cortical thickness in the left precentral cortex and the right inferior parietal, supramarginal and postcentral cortex. We found a moderating effect of gender on the association between aggressive behavior and cortical thickness in the right frontal cortex as well as in the right medial posterior cortex. While aggressive behavior was not associated with cortical surface area, we found widespread associations between aggressive behavior and decreased right hemisphere gyrification. Results were comparable across reporters.

Several studies have reported decreased amygdala volume in relation to conduct disorder, antisocial personality disorder and psychopathy (Fairchild et al., 2011; Huebner et al., 2008; Pardini, Raine, Erickson, & Loeber, 2014). In the present study, aggressive behavior was associated with a smaller amygdala volume in typically developing children. Thus, amygdala volume may be associated with aggressive behavior along a continuum within the general population. Only two studies have examined the relation between normal variation in child or adolescent aggression and amygdala volume and they did not find an association (Ameis et al., 2014; Visser et al., 2014). In our large sample, the association between aggression and amygdala volume was significant, but the effect size is small. Amygdala volume differences related to aggression may be more pronounced in clinical populations, but our findings show that the same association can be found in non-clinical groups.

Increased aggression was associated with decreased precentral, as well as inferior parietal, supramarginal, and postcentral cortical thickness. Although the precentral cortex – which is involved in motor planning and execution - has not typically been associated with aggressive behavior, several recent studies have found a relation with aggression. Precentral cortical thinning has been reported in association with psychopathy and violence (Ly et al., 2012; Narayan et al., 2007). Furthermore, activation of the precentral cortex has been associated with impulsivity in juvenile offenders and impaired response inhibition in highly aggressive male students (Pawliczek et al., 2013; Shannon et al., 2011). Structural differences of the postcentral cortex, also known as the somatosensory cortex, and the inferior partial lobule (which comprises the supramarginal and inferior parietal

cortex) have previously been reported in relation to adolescent conduct disorder as well as adult violence and antisocial behavior (Aoki, Inokuchi, Nakao, & Yamasue, 2014; Hyatt, Haney-Caron, & Stevens, 2012; Narayan et al., 2007; Tiihonen et al., 2008). Interestingly, mirror neurons have been found in the precentral cortex, postcentral cortex and inferior parietal lobule (Dushanova & Donoghue, 2010; Rizzolatti & Craighero, 2004). Mirror neurons are involved in understanding actions performed by others (Rizzolatti & Craighero, 2004). Cortical thinning in these sensorimotor regions may lead to a deficit in the understanding of others, which may cause the child to respond with aggression.

As expected, boys were more aggressive than girls (Alink et al., 2006; Borsa et al., 2013). We found a moderating effect of gender on the association between aggressive behavior and cortical thickness in a cluster covering the right precuneus, isthmus of the cingulate cortex and lingual cortex as well as in a cluster covering the right middle and superior frontal cortex. For the right medial posterior cluster, cortical thickness was positively correlated with aggressive behavior in girls, but unrelated in boys. For the right frontal cluster, a thicker cortex was related to more aggression in girls, whereas in boys, thinning of the cortex was related to more aggressive behavior. While we did not expect to find opposite results between boys and girls, a study on brain morphology of conduct disorder in adolescence has also reported opposite findings between the sexes (Fairchild et al., 2013). These opposite correlations suggest that smaller scale studies on the neurobiology of aggression should be cautious when combining males and females.

Recently, thinning of the precuneus and posterior cingulate cortex (which includes the isthmus of the cingulate cortex) has been reported in adolescents diagnosed with ODD or CD (Fahim et al., 2011; Hyatt et al., 2012; Wallace et al., 2014). Thickness of the rostral middle frontal and superior frontal cortex has been related to non-clinical conduct problems in children (Walhovd et al., 2012), and a recent meta-analysis of adult antisocial and violent behavior found that the structure and function of the DLPFC, which occupies the middle frontal gyrus, is related to aggressive behavior (Yang & Raine, 2009). In adolescent girls with conduct disorder, volume of the DLPFC was negatively correlated with aggressive conduct disorder symptoms (Fairchild et al., 2013). The precuneus, the posterior cingulate cortex, and the rostral middle frontal and superior frontal cortex are part of the default mode network (DMN) (Whitfield-Gabrieli & Ford, 2012). Activity of the DMN has been related to self-reflection, social perspective taking, moral decision-making, and future thought (Andrews-Hanna, 2012). Differences in cortical thickness in areas of the DMN may therefore be related to aggression either directly or indirectly through difficulties in self-reflection, perspective taking, and moral decision-making. In a study of default mode network connectivity in conduct disordered adolescent males with comorbid substance use disorder, Dalwani et al. (2013) found reduced activity in the middle frontal cortex, superior frontal cortex and lingual cortex in patients compared to controls. DMN activity in the precuneus, posterior cingulate cortex and lingual cortex was related to risk-taking behavior (Dalwani et al.,

2014). Investigating the role of the DMN function in relation to childhood aggression is an important area for future studies.

Childhood aggression was associated with decreased gyrification in a cluster including the right precentral cortex, extending posteriorly to the postcentral, and parietal cortex, and anteriorly to the middle and superior frontal cortex. In part of this cluster, the negative association between gyrification and aggression was found only in boys. Moreover, aggressive behavior was related to decreased gyrification in a cluster including the right lateral occipital and inferior parietal cortex. These regions partly overlap with our cortical thickness findings, and thus suggest a general rather than specific structural relation with aggressive behavior. While the association between aggressive behavior and cortical thickness was restricted to relatively small clusters, aggression related differences in gyrification were found across the entire lateral right hemisphere and thus suggest a more global effect. Considering this global effect, we tested whether the findings were also a reflection of global psychopathology. However, the associations between aggressive behavior and right hemisphere gyrification remained significant after correction for internalizing and attention problems. This provides evidence that our findings are specific markers of childhood aggression. As cortical gyrification shows its greatest growth during the third trimester of pregnancy differences in gyrification may represent the consequences of early adverse events (White, Su, Schmidt, Kao, & Sapiro, 2010).

Our cortical thickness and gyrification findings suggest that (part of) the neuroanatomical correlates of aggression may be gender-specific. Sex hormones such as testosterone have been found to affect both brain development and aggressive behavior and may thus provide an explanation for these gender-specific findings (Cunningham, Lumia, & McGinnis, 2013). Alternatively, the effect found in cortical thickness may represent a difference in maturation (Thijssen et al., 2015). Previous studies show that the social brain matures faster in girls than in boys (Mutlu et al., 2013). Moreover, several studies have suggested that children with elevated psychiatric traits (e.g. conduct problems) have a delayed or aberrant cortical maturation (De Brito et al., 2009; Dennis & Thompson, 2013). In childhood, cortical thickness increases until it reaches its peak thickness around puberty (Giedd et al., 1999). Thereafter, processes such as synaptic pruning result in cortical thinning. If the girls in our sample already show cortical thinning, the positive association between cortical thickness and aggression may be explained by delayed maturation in high aggressive girls. Indeed, in boys 3 years older than the children examined here, De Brito et al. (2009) found greater OFC, ACC and temporal lobe volume in boys with conduct problems and callous-unemotional traits compared to typically developing boys. Their post-hoc analyses indicate that their typically developing sample shows cortical decrease over age, while the boys with conduct problems either show an increase of volume over age or no relation between cortical volume and age. Due to their slower maturation compared to girls the boys in our sample may still show cortical increase. If the boys with high levels of aggressive behavior show a

delayed cortical maturation, they will have a thinner cortex compared to the low aggressive boys. However, the present study presents cross-sectional data and thus does not provide direct information on neurodevelopmental trajectories underlying aggressive behavior. Longitudinal studies are necessary to shed light on the neurodevelopmental trajectories of aggression.

Although the hippocampus has often been implicated in aggressive behavior, we did not find an association between aggression and hippocampal volume. In their study on the neuroanatomy of normal variation in aggressive behavior Visser et al. (2013) report an association between typically developing female adolescents' aggression and hippocampus volume, but in the opposite direction compared to previous research: increased hippocampal volume was related to aggression in girls. As reduced hippocampal volume has been related to aggressive behavior in individuals suffering from psychological disorders (Zetzsche et al., 2007), Visser et al. (2013) suggest that previous findings may be explained by comorbid symptoms, rather than aggression per se. Since this is the first study investigating this association in young children, differences in findings may be related to the young age of our sample. The relation between hippocampal volume and aggression in typically developing populations therefore remains an issue that requires further investigation.

Several studies on clinical and non-clinical aggression have reported reduced right ACC and OFC volume or cortical thickness in association with aggression (Ameis et al., 2014; Boes et al., 2008; Ducharme et al., 2011; Yang & Raine, 2009). Our exploratory analyses did not replicate these findings. The present study was the first structural imaging study on aggressive behavior focusing on non-clinical young children. As the (pre)frontal cortex is a brain region that matures later compared to other more primary regions of the brain (e.g. sensorimotor cortex) (Gogtay et al., 2004), the association between aggression and ACC and OFC thickness or surface area may become 'unmasked' later in life. Moreover, we used surface-based analyses to study the association between cortical morphometry and aggressive behavior. As many earlier studies have used volume-based morphometric analyses, discrepancies between the present studies and previous literature may stem from the approach used to analyze the data.

Some limitations should be noted. The present study used cross-sectional imaging data and does not provide information on development over time. Furthermore, the MRI procedure (mean age=7.9 years) was performed at a later time point than the aggressive behavior assessment (mean age=6.0 years). However, since it has been shown that aggression becomes relatively stable at age 4 (Verhulst & Van der Ende, 1995), we believe that this difference in timing of the assessment should not substantially affect the results. Moreover, analyses were corrected for age at MRI procedure and age at aggressive behavior assessment. Finally, when using a data driven approach there is always the risk of reverse inference. However, even when using such an approach, our results do mesh well with

the existing literature. Through bootstrapping we have tried to lower the risk of reporting chance findings.

In conclusion, we are the first to assess the association between brain anatomy and normal variation in childhood aggressive behavior in a large population-based sample using a multiple-informant approach. While several studies have shown associations between aggression and amygdala volume in clinical samples or in adults, we provide novel evidence that aggression is related to decreased amygdala volume also in young typically developing children. We show that childhood aggressive behavior is associated with decreased sensorimotor cortical thickness and widespread decreased right hemisphere gyrification. Moreover, aggressive behavior was associated with cortical thickness in regions that are part of the DMN, with positive associations in girls and negative associations in boys. While the associations with a priori hypothesized regions were small, larger effects were found that were widespread and suggested a more global association with brain morphology. Longitudinal studies are necessary to shed light on the developmental trajectories underlying aggression.

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SUPPLEMENTARY TEXT 2.1

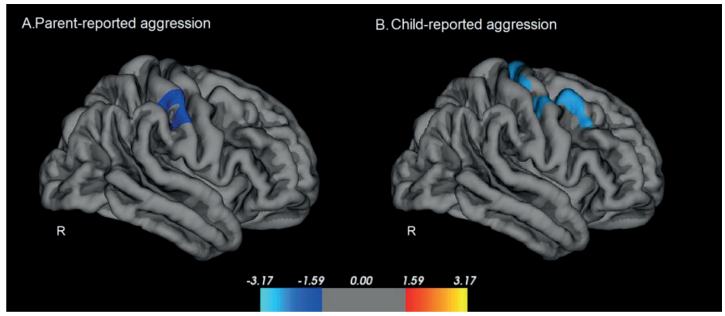
Associations between cortical thickness, cortical surface area, and gyrification and parent- and child-reported aggressive behavior

Parent-report aggressive behavior was negatively associated with cortical thickness in a cluster including the right supramarginal and postcentral cortex (1209 mm^2, max vertex X=42.9, Y=-27.6, Z=37.6, p=.004, see Supplementary Figure 2a). More child-report aggressive behavior was related to a thinner cortex in a cluster including the right precentral and caudal middle frontal cortex (1902 mm^2, max vertex X=40.2, Y=-10.9, Z=42.6, p<.001, see Supplementary Figure 2b). For both the parent and the child-report data, none of the clusters in which we found an aggression-by-gender interaction effect survived correction for multiple comparisons.

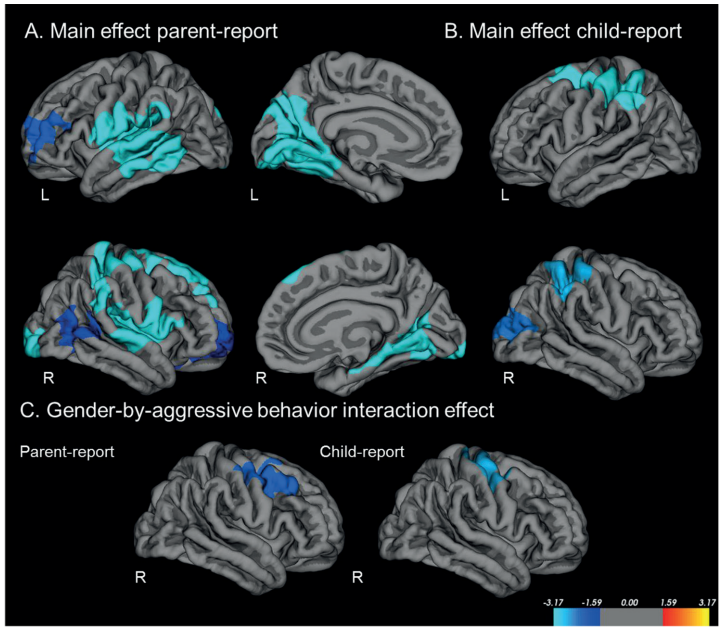
Parent-reported as well as child-reported aggressive behavior was not associated with cortical surface area. In the left hemisphere, parent-reported aggression was associated with reduced gyrification in a cluster including the pre- and postcentral gyrus, extending to the supramargincal, temporal, and insular cortex (9460 mm^2, max vertex X=-57.2, Y=-0.1, Z=-9.5, p<.001), as well as a cluster including the precuneus, cuneus, lingal and fusiform cortex, and isthmus of the cingulate cortex (7877 mm^2, max vertex X=-21.4, Y=-61.3, Z=8.9, p<.001.), and a cluster including the rostral middle frontal cortex (2440 mm^2, max vertex X=-38.1, Y=50.0, Z=-3.4, p=.01). In the right hemisphere, parent-reported aggressive behavior was associated with reduced gyrification in a cluster including the precental, postcentral, superior parietal, supramarignal, superior frontal and middle frontal cortex (6723 mm^2, max vertex X=27.7, Y=-14.4, Z=60.2, p<.001), as well as in a cluster including the insula, pre- and postcentral cortex, supramarginal and superior temporal cortex (5841 mm^2, max vertex X=37.1, Y=-13.0, Z=1.8, p<.001), and a cluster including the later occipital and lingual cortex, and precuneus (5262 mm^2, max vertex X=21.3, Y=-98.7, Z=5.3, p<.001). The fourth right hemisphere cluster included the bankssts and inferior parietal cortex (2252 mm^2, max vertex X=21.3, Y=-98.7, Z=5.3, p=.02), while a fifth cluster included the right rostral middle frontal and lateral orbitofrontal cortex (2060 mm^2, max vertex X=27.7, Y=57.8, Z=-9.5, p=.04). Gender moderated the association between parent-reported aggression and gyrification in a cluster including the right middle frontal, superior frontal, precentral, and postcentral cortex (5762 mm^2, max vertex X=32.6, Y=18.7, Z=47.2, p=.009).

Child-reported aggressive behavior was associated with reduced gyrification in a cluster including the left supramarginal, extending to the post- and precentral cortex, and caudal middle frontal and superior frontal cortex (6918 mm^2, max vertex X=-49.6, Y=-48.0, Z=44.6, p<.001). In the right hemisphere, child-reported aggression was associated with reduced gyrification in a cluster including the parietal, postcentral and supramarginal cortex (3281 mm^2, max vertex X=30.6, Y=-47.5, Z=44.4, p=.001), as well as in a cluster including the lateral occipital and parietal cortex (2830 mm^2, max vertex X=21.3, Y=-98.7, Z=5.3, p=.006).

Gender moderated the association between child-reported aggression and gyrifcation in a cluster including the right postcentral and precentral gyrus (3196 mm^2, max vertex X=27.7, Y=-14.4, Z=60.2, *p*=.001).



Supplementary Figure 2.1 | Association between cortical thickness and parent-reported and child-reported aggressive behavior. Sex, age and IQ were used as covariates (Monte Carlo corrected cluster-wise *p*<.05). Colors represent –log10 *p-value*.



Supplementary Figure 2.2 | Association between cortical gyrification and parent-reported and child-reported aggressive behavior. Sex, age and IQ were used as covariates (Monte Carlo corrected cluster-wise *p*<.05). Colors represent –log10 *p-value*.

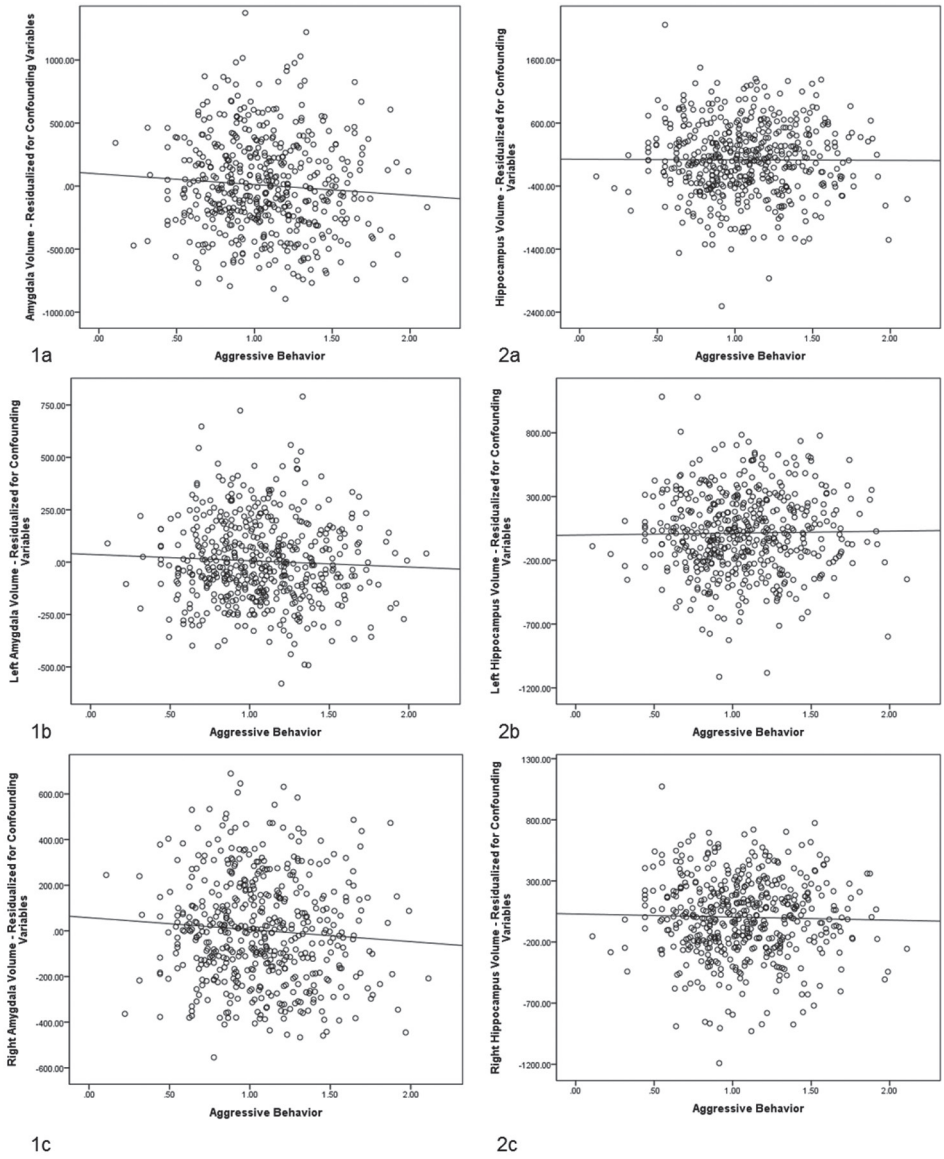
Supplementary Table 2.1 | Aggressive behavior (parental-report and child-report),amygdala and hippocampus volume (*n*=500)

			Combined			Left			Right		
			B (95% CI)	β	*part r*	B (95% CI)	β	*part r*	B (95% CI)	β	*part r*
Amygdala	Parent-report	Baseline adjusted[1]	-26.39 (-57.29; 4.51)	-.08	-.07	-9.80 (-26.00; 6.41)	-.05	-.05	-15.59 (-34.37; 3.19)	-.08	-.07
		Adjusted[2]	-48.71 (-88.99; -8.42)	-.14*	-.10	-17.37 (-38.41; 3.67)	-.10	-.07	-31.15 (-55.62;-6.69)	-.15*	-.11
	Child-report	Baseline adjusted[1]	-13.17 (-49.44; 23.10)	-.03	-.03	-3.87 (-22.88; 15.85)	-.02	-.02	-8.69 (-30.73; 13.35)	-.04	-.04
		Adjusted[2]	-16.22 (-52.99; 20.54)	-.04	-.04	-6.30 (-25.49; 12.88)	-.03	-.03	-9.45 (-31.85; 12.95)	-.04	-.04
Hippo-campus	Parent-report	Baseline adjusted[1]	18.77 (-28.27; 65.81)	.03	.03	14.12 (-11.66; 39.90)	.05	.05	4.15 (-21.98; 30.27)	-.00	.01
		Adjusted[2]	5.13 (-56.38; 66.65)	.01	.01	4.54 (-28.98; 38.05)	.02	.01	0.50 (-33.71; 34.70)	.01	.00
	Child-report	Baseline adjusted[1]	-3.99 (-59.14; 51.15)	-.01	-.01	2.99 (-27.34; 33.21)	.01	.01	-7.29 (-37.90; 23.32)	-.02	-.02
		Adjusted[2]	-15.57 (-71.38; 40.23)	-.03	-.03	-4.97 (-35.40; 25.46)	-.02	-.02	-10.42 (-41.53; 20.69)	-.03	-.03

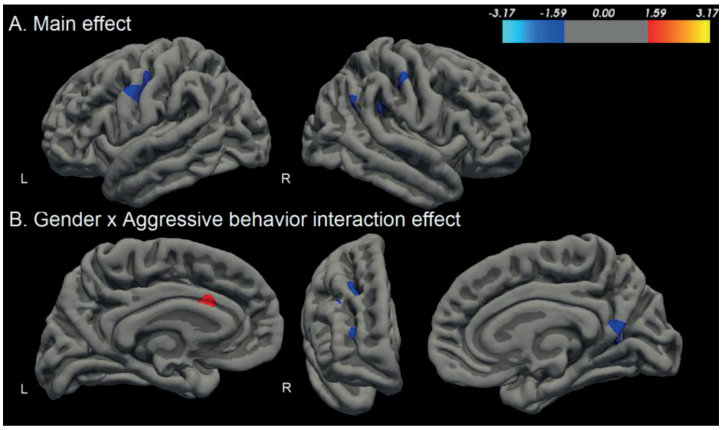
Note. * $p<05$ ** $p<.01$*** $p<.001$. Amygdala and hippocampus volume were corrected by TBV.

[1] Adjusted for gender and age

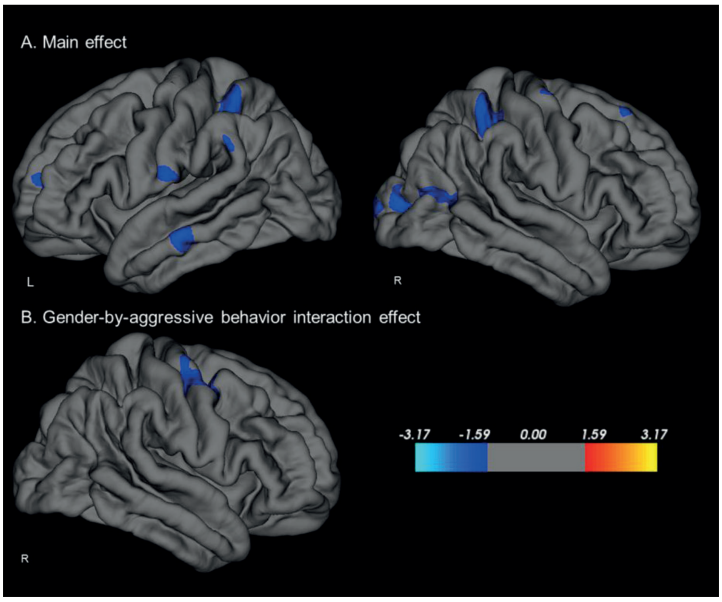
[2] Adjusted for gender, age, IQ, gestational age, ethnicity, handedness, attention problems, internalizing problems, prosocial behavior, image quality, maternal education, parental psychopathology, maternal smoking during pregnancy



Supplementary Figure 2.3 | Scatterplot of the association between aggressive behavior and amygdala **1a**. amygdala volume; **1b**. left amygdala volume; **1c**. right amygdala volume; **2a.** hippocampal volume; **2b.** left hippocampus volume; **2c.** right hippocampus volume. Amygdala and hippocampal volumes were residualized for total brain volume and confounding variables.



Supplementary Figure 2.4 | Averaged results of the bootstrapped association between cortical thickness and aggressive behavior. **A.** Aggressive behavior was related to a thinner cortex in a cluster including the left precentral cortex and a cluster including the right inferior parietal, supramarginal, and postcentral cortex. **B.** Gender moderates the associations between aggressive behavior and cortical thickness in a cluster including the left ACC, in a cluster including the right middle and superior frontal cortex and in a cluster including the right precuneus. Colors represent –log10 *p-value*.



Supplementary Figure 2.5 | Averaged results of the bootstrapped association between cortical gyrification and aggressive behavior. **A.** Aggressive behavior was related to a decreased gyrification in the left middle temporal, parietal, postcentral and middle frontal cortex as well as the in the right parietal, lateral occipital, precentral and middle frontal cortex. **B.** Gender moderates the associations between aggressive behavior and cortical gyrification in a cluster including the right precentral gyrus. Colors represent –log10 *p-value*.

Chapter 3

Cortical thickness and prosocial behavior in school-age children: a population-based MRI study

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ABSTRACT

Objective: Prosocial behavior plays an important role in establishing and maintaining relationships with others and thus may have important developmental implications. This study examines the association between cortical thickness and prosocial behavior in a population-based sample of 6- to 9-year old children.

Methods: The present study was embedded within the Generation R Study. MR scans were acquired from 464 children whose parents had completed the prosocial scale of the Strengths and Difficulties Questionnaire. To study the association between cortical thickness and prosocial behavior we performed whole-brain surface-based analyses.

Results: Prosocial behavior was related to a thicker cortex in a cluster that covers part of the left superior frontal and rostral middle frontal cortex ($p<.001$). Gender moderated the association between prosocial behavior and cortical thickness in a cluster including the right rostral middle frontal and superior frontal cortex ($p<.001$) as well as in a cluster covering the right superior parietal cortex, cuneus and precuneus ($p<.001$).

Conclusions: Our results suggest that prosocial behavior is associated with cortical thickness in regions related to theory of mind (superior frontal cortex, rostral middle frontal cortex cuneus, and precuneus) and inhibitory control (superior frontal and rostral middle frontal cortex).

INTRODUCTION

Prosocial behavior, defined as voluntary behavior intended to benefit another person, plays a critical role in establishing and maintaining relationships with others and thus may have important developmental implications (Eisenberg, Fabes, & Spinrad, 2007). In children, prosocial tendencies are positively related to peer acceptance and academic achievement (Layous, Nelson, Oberle, Schonert-Reichl, & Lyubomirsky, 2012; Shlafer, McMorris, & Sieving, 2012). Moreover, while its relationship with antisocial behaviour is complex, prosocial behavior may counterbalance aggressive and externalizing proclivities (Caprara, Barbaranelli, Pastorelli, Bandura, & Zimbardo, 2000; Fabes, Carlo, Kupanoff, & Laible, 1999; Hastings, Zahn-Waxler, Robinson, Usher, & Bridges, 2000; Hawley, 2003). As several childhood disorders are characterized by a lack of prosocial behavior (e.g. attention deficit hyperactivity disorder, conduct disorder), studying the neuroanatomical correlates of prosocial behavior may provide important insights on normal behavior and psychopathology. Nevertheless, neuroanatomical correlates of prosocial behavior have only rarely been studied, especially in children. Here, we examine the association between cortical thickness and prosocial behavior in a large population-based sample of 6- to 9-year old children.

Prosocial behavior develops early in life. Before reaching the age of 2 years, infants are capable of carrying out various prosocial acts, such as helping, comforting, and sharing (Eisenberg et al., 2007; Schmidt & Sommerville, 2011). From infancy onwards, prosocial behavior has been found to increase (for a meta-analysis, see Eisenberg et al., 2007). Age-related changes in prosocial behavior may partly stem from the emergence and development of two related yet distinct abilities, namely cognitive empathy or theory of mind (ToM, the ability to represent others' mental states), and emotional empathy (the capacity to understand or share others' emotions and feelings). Several studies have shown that a child's developing ability to understand others' mental states and/or emotions is predictive of prosocial behavior (Eggum et al., 2011; Eisenberg et al., 2007; Moore, Barresi, & Thompson, 1998). Moreover, emotional empathy may be one of the most important motivations for prosocial behavior.

Current knowledge on the neurobiology of prosocial behavior predominantly stems from functional MRI studies in adults. While studies directly measuring prosocial brain activity are scarce, several imaging studies have related neural activation in response to related processes such as empathy to prosocial behavior. Although these studies do not provide information on the neurobiology of prosocial behavior itself, they may provide insights on neural processes related to prosociality. Masten, Morelli and Eisenberger (2011) showed that in adults, activation of the medial prefrontal cortex (MPFC) and the right anterior insula in response to the observation of social exclusion predicted later prosocial behavior towards the excluded individual. When this study was repeated in young adolescents, activity in the anterior insula, posterior cingulate cortex and precuneus was predictive of prosocial

behavior (Masten, Eisenberger, Pfeifer, & Dapretto, 2010). Moreover, activity in the MPFC in response to induced empathic concern also predicts other forms of prosocial behavior, such as donating and helping (Mathur, Harada, Lipke, & Chiao, 2010; Rameson, Morelli, & Lieberman, 2012; Waytz, Zaki, & Mitchell, 2012).

A more direct assessment of prosocial neural activity can be inferred from imaging studies on donating (Kuss et al., 2013; Moll et al., 2006; Telzer, Masten, Berkman, Lieberman, & Fuligni, 2011). In these donating studies, participants are presented with an offer that affects both the participant and another individual or charitable organization. Similar to receiving a monetary reward, making a donation has been associated with activity in the nucleus accumbens, ventral tegmental area and striatum, areas involved in reward processing (Kuss et al., 2013; Moll et al., 2006). Costly-donations (monetary loss for participant and monetary gain for other party) have been associated with activity in the MPFC and the cuneus, areas involved in ToM, and the dorsolateral prefrontal cortex (DLPFC), which has previously been related to inhibitory control (Telzer et al., 2011). Activation of the DLPC has also been related to different forms of prosocial behavior. In a repetitive transcranial magnetic stimulation (rTMS) study, Balconi and Canavesio (2014) asked their participants whether they wanted to provide help to the agent in a video clip. High-frequency stimulation of the DLPFC was associated with increased helping.

A recent study on the neural correlates of prosocial behavior in infancy has shown that infant's resting state brain activation asymmetries as measured by electroencephalography (EEG) may be related to early forms of prosocial behavior (Paulus, Kuhn-Popp, Licata, Sodian, & Meinhardt, 2013). While greater left frontal cortical activation was associated with comforting, greater right temporal activation was associated with infant's instrumental helping.

Studies on the neurological background of William's syndrome may also provide important information on brain morphology of prosocial behavior. William's syndrome is a neurodevelopmental disorder which is characterized by hypersociability and heightened empathy. Several fMRI studies have shown abnormalities in amygdala and prefrontal regulation which may partly account for this specific social phenotype (Capitao et al., 2011; Mimura et al., 2010).

While functional imaging studies provide useful information on short-lived brain-behavior associations, structural imaging studies may provide information on more long-term associations between the brain and behavior. Cortical thickness is a brain morphometric measure used to describe the combined thickness of the layers of the cerebral cortex and thus may be associated with cognitive abilities. Cortical development is characterized by a prepubertal increase in cortical thickness, followed by (post)pubertal cortical thinning. Regions associated with more primary functions develop first (motor cortex, visual cortex), followed by higher -order association cortices (prefrontal cortex, temporal cortex) (Giedd et al., 1999; Gogtay et al., 2004). Differences in cortical thickness and cortical development

have been found in relation to several psychiatric disorders (e.g. Fairchild et al., 2013), and recently, several studies have shown that also normal variation in behavior is related to cortical thickness (e.g. Walhovd, Tamnes, Ostby, Due-Tonnessen, & Fjell, 2012). Furthermore, cortical development may vary by gender. For example, the superior temporal cortex, a region associated with ToM, may develop faster in girls than in boys (Mutlu et al., 2013) Gender differences have also been reported in relation to prosocial behavior. In general, girls are reported to be more prosocial than boys, although the strength of the gender difference depends on the type of prosocial behavior under investigation (Baillargeon et al., 2011; Eisenberg et al., 2007).

To our knowledge, structural brain differences related to prosocial behavior have only been investigated in a study of social-emotional development in a cohort of children born very preterm (Rogers et al., 2012). This study reported a positive relation between bifrontal diameter at term equivalent age and prosocial behavior in 5-year old boys. As this study only looked at fetal brain metrics in preterm children, it is still unclear whether similar associations can be found postnatal or in children born at term.

The present paper aims to explore the association between cortical thickness and prosocial behavior in a population-based sample of 6- to 9-year old children. As previous research suggests a complicated relationship between prosocial and antisocial behavior, studying the neuroanatomical correlates of prosocial behavior in the absence of antisocial behavior might provide limited results (Fabes et al., 1999; Renouf et al., 2010; Veenstra et al., 2008). Therefore, the association between prosocial behavior and cortical thickness will be controlled for aggressive behavior. Although functional MRI studies suggest involvement of the frontal cortex in prosocial behavior, only few imaging studies have looked at brain activity in response to prosocial behavior directly. In addition, the association between cortical thickness and prosocial behavior has not previously been examined. Therefore, we will use a data driven approach, examining the association between cortical thickness and prosocial behavior across the entire cortex. As gender differences in prosocial behavior and cortical development have been well characterized in the literature (Baillargeon et al., 2011; Eisenberg et al., 2007; Mutlu et al., 2013; Raznahan et al., 2010), we expect that the association between cortical thickness and prosocial behavior may be moderated by gender.

METHODS

Participants

Participants were recruited from the Generation R Study, a population-based prospective cohort from early fetal life onwards in Rotterdam, the Netherlands (Jaddoe et al., 2012; Tiemeier et al., 2012). From a total of 703 eligible children, 149 children (21%) did not have

a prosocial score. Seventy children (10%) were excluded due to poor quality imaging data. Due to random selection, this sample includes seven twin pairs and six sibling pairs. Twin pairs were excluded from the analyses, as well as a randomly selected child from each sibling pair. Consequently, analyses were performed on 464 6- to 9-year old children (234 boys) for whom data on prosocial behavior and good quality T1-weighted imaging data were available. This study was approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was provided by the parents.

Measures

Prosocial behavior

The prosocial scale of the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997; Paap et al., 2013) was used to assess prosocial behavior. This scale consists of five items, e.g. 'My child often spontaneously offers to help other' and 'My child is considerate of other people's feelings', scored on a 3-point Likert scale (1 = not true, 2 = somewhat true, 3 = certainly true; α=.73, *M*=13.27, *SD*=1.80). A meta-analysis on the psychometric properties of the SDQ shows acceptable test-retest reliability (*r*=.65) and construct validity (Stone, Otten, Engels, Vermulst, & Janssens, 2010). Other studies have shown its discriminant validity by showing negative associations with the problem scales of the SDQ or Child Behavior Checklist (Gomez, 2014; Hawes & Dadds, 2004; Theunissen, Vogels, de Wolff, & Reijneveld, 2013). The SDQ prosocial scale was completed by the child's primary caregiver when the child was on average 6 years of age. Because the distribution of scores was negatively skewed, scores were inverted and square root transformed (Tabachnick & Fidell, 2007). To assure that high scores would correspond to more prosocial behavior, the transformed scores were inverted.

Aggressive behavior

The primary caregiver completed the Child Behavior Checklist 1½ – 5 (CBCL, Achenbach & Rescorla, 2000) when the children were on average 6 years of age (α=.84, *M*=5.10, *SD*=4.23). The CBCL can be scored on eight scales, including the Aggressive Behavior scale that was used in the present study. This scale consists of 19 items, such as 'Defiant', scored on a 3-point Likert scale (0 = not true, 1 somewhat or sometimes true, 2 = very true or often true). For nine children, Aggressive Behavior scores at age 6, but not at age 3, were missing. For these cases, Aggressive Behavior scores collected at age 3 were imputed. For seven children, no Aggressive Behavior score was available. For these children, the median score was imputed. Because the distribution of scores was positively skewed, scores were square root transformed (Tabachnick & Fidell, 2007).

Covariates

The covariates specified below were considered factors that could confound the association between cortical thickness and prosocial behavior. Information on gender and date of birth were obtained from midwives and hospital registries. Ethnicity, family income, and maternal education level were assessed through questionnaires. Ethnicity was defined according to the Dutch standard classification criteria (Statistics & Netherlands, 2004) and categorized as 'Western' (Dutch, other European, North-American and Oceanian) or 'non-Western' (Turkish, Moroccan, Indonesian, Cape Verdean, Surinamese and Antillean). Maternal education level was defined as the highest completed education and was categorized into primary (no or primary education), secondary (lower and intermediate vocational training), and higher (higher vocational education and university) education. Handedness was measured using a modified version of the Edinburgh Handedness Inventory (Oldfield, 1971). Intelligence was estimated from the Mosaics and Categories subtest of the Snijders-Oomen Non-Verbal Intelligence Test -Revised (Niet-verbale Intelligentie Test – Revisie SON-R 2½ – 7) (Tellegen, Winkel, Wijnberg-Williams, & Laros, 2005). Executive functioning was measured using the Auditory Attention and Response Set task of the NEPSY II (Brooks, Sherman, & Strauss, 2010). We generated an executive functioning score by computing the sum of the standardized total scores of the two tasks. Parental psychopathology was measured using the anxiety and depression scales of the Brief Symptom Inventory (BSI) (Derogatis & Melisaratos, 1983). BSI data were collected prenatally and again when the child was three years of age. Mean depression and anxiety scores were computed as the mean of these two time points. When either prenatal or postnatal BSI data was missing, the available BSI score was used as a measure of parental depression or anxiety. Models were adjusted for covariates that generated a change in predictor effect estimate of 5% or more.

Structural Magnetic Resonance Imaging

A description of the neuroimaging component of the Generation R Study has been described elsewhere (White et al., 2013). Briefly, children were first familiarized with the scanning environment through the use of a mock scanning session. During this session the important features of the actual MRI session were simulated. MRI scanning was performed on a GE Discovery MR 750 3 T scanner (General Electric, Milwaukee, MI, USA). T1-weighted inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence was obtained using an 8 channel head coil with the following parameters: TR=10.3 ms, TE=4.2 ms, TI=350 ms, NEX=1, flip angle=16°, readout bandwidth= 20.8 kHz, matrix 256 x 256, imaging acceleration factor of 2, and an isotropic resolution of 0.9x0.9x0.9 mm^3. The children were able to watch a film or listen to a CD during the acquisition.

Image Processing

Cortical reconstruction and volumetric segmentation was performed with the Freesurfer image analysis suite 5.1 (http://surfer.nmr.mgh.harvard.edu/). The technical details of these procedures are described in prior publications (Dale, Fischl, & Sereno, 1999). Briefly, this process included the removal of non-brain tissue using a hybrid watershed/ surface deformation procedure, automated Talairach transformation into standard space, segmentation of the subcortical white matter and deep gray matter volumetric structures intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction , and surface deformation.

Once the cortical models were complete, a number of deformable procedures were performed for further data processing and analysis. These included surface inflation (Fischl, Sereno, & Dale, 1999), registration to a spherical atlas that utilized individual cortical folding patterns to match cortical geometry across subjects (Fischl, Sereno, Tootell, & Dale, 1999), and the parcellation of the cerebral cortex into units based on gyral and sulcal structure (Desikan et al., 2006). Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface (Fischl & Dale, 2000). The surface based map was smoothed with a 10 mm full-width half-maximum (FWHM) Gaussian kernel prior to the surface based analyses (Du et al., 2007; El Marroun et al., 2013). Numerous studies using Freesurfer in typical and atypical developing school-aged children are available (El Marroun et al., 2013; Juuhl-Langseth et al., 2012).

Image quality

Following MRI acquisition, unprocessed T1 images were visually inspected at the scan site. Raters were instructed to check the images for movement and scanner artifacts by evaluating a number of different regions within the brain. This included observing whether the boundaries between gray and white matter were clearly visible, examining how much detail could be seen of the cerebellum (foliation), and whether specific landmarks, (i.e., corpus callosum, hippocampus, caudate, etc.) were clearly (or unclearly) defined. Images were rated on a 6-point scale (unusable, poor, fair, good, very good, excellent). Images were then processed through the FreeSurfer pipeline after which segmentation quality was evaluated. When assessing segmentation quality, we assessed whether segmentation followed visual gray and white matter characteristics and looked for artifacts in the 3D output, such as holes and bridges. Images were rated on a 6-point scale (not constructed, poor, fair, good, very good, excellent). Unprocessed images that were rated as unusable or poor were excluded from the analyses (*N*=25) as well as images that could not be processed by FreeSurfer or the images in which the segmentation quality was rated as poor (*N*=45).

Statistical analyses

Depending on the distribution of the data, missing values for the covariates (2% – 7%) were imputed using the group mean or median, or in case of categorical variables, the mode.

Analyses of the demographic and SDQ data were performed using *t*-tests and Pearson correlations coefficients. As the association between cortical thickness and prosocial behavior has not previously been studied, we performed vertex-wise exploratory analyses of cortical thickness across the entire cortex. Furthermore, as the boundaries of a priori-defined regions of interest (ROIs) may not exactly correspond to boundaries of regions associated with prosocial behavior, performing whole-brain surface based analyses may provide more detailed information on the location of an association. For these purposes, FreeSurfer's QDEC was used (www.surfer.nmr.mgh.harvard.edu). Regions for which cortical thickness was significantly related to prosocial behavior were determined using general linear models (GLMs) with age, gender and IQ as covariates. We also examined whether gender moderated the association between cortical thickness and prosocial behavior. To correct for the effect of multiple comparisons, a whole-brain Monte Carlo analysis was performed using 10,000 iterations and a cluster size of 1.3 (*p-value*≤.05).

A surface-based ROI was created for brain areas that were significantly related to prosocial behavior and for regions in which there was a significant gender-by-prosocial behavior interaction effect. The ROIs were utilized to extract the mean cortical thickness within the specific ROI for every participant. Cortical thickness of the ROIs was then residualized for total brain volume (TBV). Hierarchical linear regression models were used on the individual cortical thickness measures to further investigate the relationship with prosocial behavior and adjustment for aggression (gender, age and cortical thickness in the first step, covariates in the second step, aggressive behavior in the third step). Only for the ROIs in which there was a significant moderating effect of gender, IQ, maternal education and parental psychopathology generated a change in predictor effect estimate of 5% or more. Therefore these variables were used as covariates. Age-by-cortical thickness, gender-by-age-by-cortical thickness interaction, and aggression-by-cortical thickness interaction effects were also tested. As these interaction effects were not significant, they were not reported.

To confirm our primary findings, we bootstrapped the GLM analysis using 500 samples of 200 participants. For each voxel, *p-values* were averaged to generate an overall result.

RESULTS

The demographic characteristics of the participants can be found in Table 3.1. Boys and girls were similar in age at the SDQ assessment ($t(462)=-1.52$, $p=.13$) and age at the MRI procedure ($t(462)=-1.57$, $p=.12$). A positive correlation between age at SDQ assessment and

prosocial behavior was found, *r*=.10, *p*=.037. Furthermore, as expected, gender of the child was related to prosocial behavior, *t*(462)=3.96, *p*<.001. Girls (*M*=13.60, *SD*=1.67) showed more prosocial behavior than boys (*M*=12.96, *SD*=1.88). Prosocial behavior was negatively correlated with aggression, *r*=-.18, *p*<.001.

Table 3.1 | Sample Characteristics

Child characteristics	**N=464**	***M (SD)*/N(%)**	**Parental characteristics**	**N=464**	***M (SD)*/N(%)**
Gender	464		Reporter psychopathology	450	
Boy		234 (50.4)	Anxiety		1.50 (2.55)
Ethnicity	464		Depression		1.46 (2.84)
Western		377 (81.3)	Maternal education level	464	
Non-western		87 (18.8)	Primary school		12 (2.6)
Aggressive Behavior	448	5.10 (4.23)	Secondary education		190 (40.9)
IQ	433	103.70 (14.02)	Higher education		262 (56.5)
Attention and Executive Functioning score	454	0.07 (0.88)	Family net income	434	
SDQ filled out by	454		<1200 euro		24 (5.2)
Mother		417 (90.0)	1200 – 2400		86 (18.5)
Father		32 (6.9)	>2400 euro		324 (69.8)
Together		5 (1.1)			
SDQ prosocial	464	13.28 (1.80)			
Age SDQ	464	6.22 (0.49)			
Age MRI	464	7.74 (0.96)			

Association between prosocial behavior and cortical thickness

After Monte Carlo correction for multiple testing, the GLM testing for the relationship between cortical thickness and prosocial behavior revealed one significant cluster covering part of the left superior frontal and rostral middle frontal cortex (1600 mm^2, max vertex X=-14.6, Y=59.4, Z=13.8, *p*=.0001, see Figure 3.1). A higher score for prosocial behavior was related to a thicker cortex in this cluster. Gender moderated the association between prosocial behavior and cortical thickness in a cluster covering the right superior parietal cortex, extending to the cuneus, and precuneus (2132 m^2, max vertex X=11.5, Y=-89.5, Z=21.1, *p*=.0001, see Figure 3.1), as well as in a cluster including the right rostral middle frontal and superior frontal cortex (2002 m2, max vertex X=34.4, Y=49.8, Z=7.7, *p*=.0001, see Figure 3.1).

To examine whether the association between the frontal ROI (predictor) and prosocial behavior (outcome) could be explained by variance in aggressive behavior, a hierarchical linear regression model was used. Results of this analysis can be found in Table 3.2. Cortical

thickness of the left frontal ROI was significantly related to prosocial behavior, β=.18, *p*<.001, and inclusion of aggressive behavior did not affect this association.

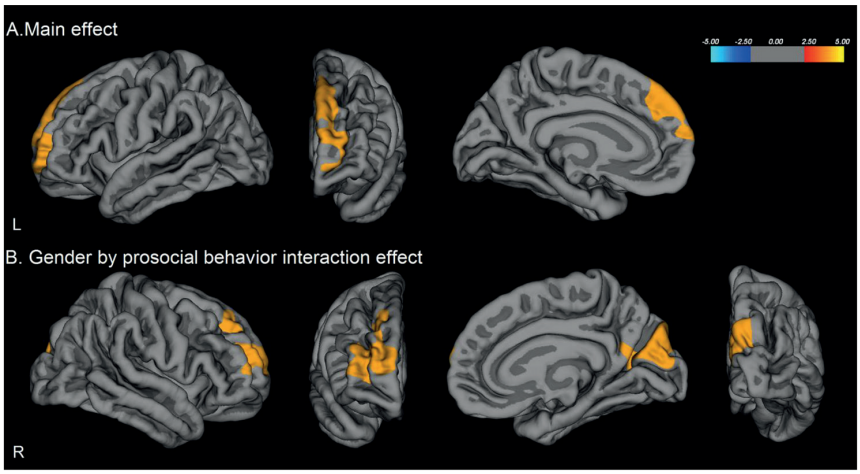

Figure 3.1 | A. Relation between cortical thickness and prosocial behavior. Sex, age, and IQ were included as covariates. Cortical thickness was positively related to prosocial behavior in one cluster covering part of the left superior frontal, and rostral middle frontal cortex (Monte Carlo corrected cluster-wise *p*<.05). Colors represent –log10 *p-value*. **B.** A moderating effect of gender was found for a cluster including the right rostral middle frontal, and superior frontal cortex and for a cluster including the right superior parietal cortex, cuneus and precuneus. Colors represent –log10 *p-value*.

Table 3.2 | Relationship between Extracted Brain Regions and Prosocial Behavior

	Prosocial behavior	
	B (95% CI)	**β**
Model1[a]	0.39 (0.20; 0.58)	.20***
Model2[b]	0.40 (0.21; 0.58)	.20***

Note. * *p*<.01, ** *p*<.01, *** *p*<.001. Cortical thickness was residualized for TBV. ROI covers part of the left superior frontal cortex and rostral middle frontal cortex

[a] Analyses were corrected for age, IQ, and gender

[b] Analyses were corrected for age, IQ, gender, aggressive behavior

For regions in which there was a significant gender by prosocial interaction effect, analyses were repeated for boys and girls separately (Table 3.3). In girls, we found a negative association between cortical thickness of the right posterior ROI and prosocial behavior,

β=-.20, *p*<.01. In boys, this association was positive, β=.18, *p*<.01. For the right frontal ROI we found that a thicker cortex was associated with more prosocial behavior in boys, β=.20, *p*<.01. In girls, the association between cortical thickness and prosocial behavior in this region was negative, β=-.13, *p*=.04.

To confirm our primary findings, we bootstrapped the GLM analysis using 500 samples of 200 participants. Supplementary Figure 3.1 shows a cortical map of the averaged *p-values* across the 500 bootstraps. All clusters were confirmed.

Table 3.3 | Gender Moderating Effects in Extracted Brain Regions

	Cortical thickness x Gender		Boys		Girls	
	B (95% CI)	**β**	**B (95% CI)**	**β**	**B (95% CI)**	**β**
R posterior ROI						
Model1[a]	0.95 (0.50; 1.40)	.25***	0.48 (0.12; 0.83)	.17**	-0.49 (-0.78; -0.20)	-.21***
Model2[b]	0.99 (0.53; 1.44)	.26***	0.52 (0.16; 0.89)	.19**	-0.50 (-0.79; -0.21)	-.20***
Model3[c]	0.94 (0.49; 1.39)	.25***	0.49 (0.13; 0.84)	.18**	-0.49 (-0.77; -0.20)	-.20**
R frontal ROI						
Model1[a]	0.79 (0.39; 1.20)	.24***	0.47 (0.17; 0.77)	.20**	-0.31 (-0.58; -0.03)	-.14*
Model2[b]	0.80 (0.39; 1.21)	.24***	0.48 (0.18; 0.78)	.20**	-0.31 (-0.59; -0.04)	-.15*
Model3[c]	0.76 (0.36; 1.17)	.23***	0.48 (0.18; 0.77)	.20**	-0.28 (-0.56; -0.01)	-.13*

Note. * *p*<.05, ** *p*<.01, *** *p*<.001. Cortical thickness was residualized for TBV. ROI covers part of the right superior parietal cortex, cuneus and precuneus.

[a] Analyses were corrected for age, IQ (and gender)

[b] Analyses were corrected for age, (gender), IQ, parental psychopathology

[c] Analyses were corrected for age, (gender), IQ, parental psychopathology, aggressive behavior.

DISCUSSION

The present study examined the association between cortical thickness and prosocial behavior in a population-based sample of 6- to 9-year old children. More prosocial behavior was associated with a thicker cortex in a cluster that covers part of the left superior frontal and rostral middle frontal cortex. We found a significant gender-by-cortical thickness interaction effect in a cluster including the right superior parietal cortex, cuneus and precuneus as well as in a cluster including the right rostral middle frontal and superior frontal cortex. For both clusters, prosocial behavior was related to a thicker cortex in boys. In girls, we found a negative association between prosocial behavior and cortical thickness in the right frontal and right posterior ROI. Controlling for aggression did not change the association between prosocial behavior and cortical thickness.

The MPFC and the lateral prefrontal cortex (LPFC) -which involve the medial and lateral anterior part of the superior frontal cortex as well as the middle frontal cortex- have often

been implicated in ToM or mentalizing (for a review on neuroanatomical correlates of ToM see Carrington & Bailey, 2009; Denny, Kober, Wager, & Ochsner, 2012; Singer, 2006). ToM plays an important role in prosocial behavior. Several studies suggest that a child's emerging and developing ToM is predictive of prosocial development (Eggum et al., 2011; Eisenberg et al., 2007; Moore et al., 1998). Moreover, previous functional MRI studies have shown that regions associated with mentalizing, including the MPFC and precuneus, are more activated in response to the observation of social exclusion and sad images, and that this activation is predictive of later prosocial behavior (Masten et al., 2010; Masten et al., 2011; Rameson et al., 2012). Although these latter studies did not measure prosocial brain activation directly, they do suggest that MPFC activation is related to prosociality. Our findings of an association between cortical thickness of the superior frontal and rostral middle frontal cortex and prosocial behavior support these results.

Besides ToM, the LPFC has been associated with executive functions, such as inhibitory control (Durston et al., 2002). In a recent imaging study on prosocial behavior, Telzer et al. (2011) reported activation of the left DLPFC in response to costly-donations. Moreover, this same study as well as other donating studies have shown that individuals need more time to make a costly prosocial decision compared to a non-costly decision (Moll et al., 2006; Telzer et al., 2011). This increase in decision time may implicate that the decision to donate was preceded by some form of cognitive conflict. Telzer et al. therefore suggest that the activation in the DLPFC may not be related to the prosocial act itself, but rather to the suppression or inhibition of a selfish response which may have preceded the prosocial act. Several studies on prosocial behavior in children have found positive associations between inhibitory control or self-regulation and prosociality (Carlo, Crockett, Wolff, & Beal, 2012; Hughes, White, Sharpen, & Dunn, 2000; Moore et al., 1998). Although the association between cortical thickness of the lateral superior frontal and rostral middle frontal cortex and prosocial behavior in our study was not affected by correction for our measure of executive functioning, our results may support these findings.

Similar to previous studies, our results suggest that girls exhibit more prosocial behavior than boys. While a thicker left frontal cortex was associated with more prosocial behavior in both boys and girls, the association between cortical thickness and prosocial behavior in the right frontal cortex was moderated by gender. Moreover, also in a cluster covering the superior parietal cortex, cuneus and precuneus the association between cortical thickness and prosocial behavior was different for boys and girls. Like the medial superior frontal cortex, the cuneus and precuneus have been implicated in ToM (Carrington & Bailey, 2009; Denny et al., 2012). In their study on the neural basis of empathy in early adolescence, Masten et al. (2010) found engagement of the superior frontal cortex, and precuneus during the observation of peer rejection. There was a negative association between precuneus activation and later prosocial behavior. Moreover, activation in the cuneus has been reported in relation to making a costly-donation (Telzer et al., 2011). The association

between prosocial behavior and cortical thickness in this region may converge with these findings.

For both the right frontal and the right posterior ROI, a thicker cortex was associated with more prosocial behavior in boys, while a thinner cortex was associated with more prosocial behavior in girls. These results seem puzzling. However, sex hormones such as testosterone affect brain development but are also related to prosocial behavior and may thus (partly) account for the gender differences presented here (Boksem et al., 2013; Cunningham, Lumia, & McGinnis, 2013). Alternatively, the social brain appears to mature faster in girls than in boys (Mutlu et al., 2013). Moreover, several studies have shown that children suffering from psychiatric disorders have a delayed or aberrant cortical maturation (Dennis & Thompson, 2013). If this also holds true for children who show low levels of prosocial behavior, the negative association between cortical thickness and prosocial behavior in girls and the positive association between prosocial behavior and cortical thickness in boys may be explained by differences in maturation. In childhood, cortical thickness increases until it reaches its peak thickness around puberty (Giedd et al., 1999). Thereafter, processes such as synaptic pruning and continued myelination result in cortical thinning seen on MRI. If the girls in our sample already show cortical thinning, the negative association between cortical thickness and prosocial behavior may be explained by delayed maturation in girls with less prosocial behavior. If boys show a slower maturation than girls, the boys in our sample may continue to show an increase in cortical thickness. If the boys with low levels of prosocial behavior show a delayed cortical maturation, they will have a thinner cortex compared to boys with more prosocial behavior. However, in post-hoc analyses we tested age-by-gender-by-prosocial behavior interaction effects on cortical thickness in the right posterior and frontal ROI. As these three-way interaction effects were not significant, we do not find evidence to support this explanation. Alternatively, we may not have enough statistical power to find a three-way interaction effect. As the present study was a cross sectional study, it only provides a snapshot in time and not direct information on developmental trajectories. Longitudinal studies are necessary to shed light on the neuroanatomical trajectories underlying prosocial behavior.

In their paper on the determinants of children's donating to charity, Van IJzendoorn, Bakermans-Kranenburg, Pannebakker, and Out (2010) reported that the situation was a more powerful determinant of prosocial behavior than genetics, attachment, temperament or parenting. Since the present study reports associations between prosocial behavior and cortical thickness, our findings suggest that prosocial behavior may not be solely determined by situational characteristics, but may also be related to individual differences in neuroanatomy.

Some limitations of this study should be noted. Although observational data would be preferable, the present study used questionnaire data to assess prosocial behavior. Questionnaire data tends to be more biased compared to observational data. Parental

psychological state may affect the way parents experience and thus report their child's behavior. Parents may report more stereotypic gender roles, and thus view girls as more prosocial than boys. Future studies may want to explore associations between brain morphology and observational measures of prosocial behavior. As only few studies have investigated structural correlates of prosocial behavior, functional imaging studies have been used to interpret the present results. While the relation between function and structure is complex (Strenziok et al., 2011; Tremblay, Dick, & Small, 2013), interpreting structural results based on functional studies remains speculative. Moreover, whereas the functional studies have often used observational measures of prosocial behavior, we used a parent report measure of prosociality. As different measures of prosocial behavior were used, results may not be easily comparable. Finally, when using a data driven approach there is always the risk of reverse inference. However, even when using such an approach, our results do mesh well with the existing literature. Through bootstrapping we have tried to lower the risk of capitalizing on chance.

It is widely assumed that brain morphology influences behavior. However, behavior can also influence brain anatomy. One well-known example of behavior influencing anatomy is the study of London taxi drivers, who showed an increase in the size of the hippocampus related to their memorization of the map of London (Maguire et al., 2000). In our cross-sectional neuroimaging study, we cannot test the causal direction of the association between cortical thickness and prosocial behavior. However, if prosocial behavior influences brain morphology and vice-versa, the finding of an association between prosocial behavior and cortical thickness may be relevant for intervention purposes. More research is needed before intervention programs may profit from the finding that prosocial behavior is related to cortical thickness.

CONCLUSIONS

The present study is the first to examine the association between brain morphology and prosocial behavior in a population-based sample. Our results suggest that prosocial behavior is associated with cortical thickness in areas that have been related to theory of mind and executive functioning (e.g. inhibitory control). These findings are in accordance with developmental studies showing association between theory of mind or executive function and prosocial behavior. However, as the present study used a parent-report measure of prosocial behavior, observational studies may be necessary to confirm the present findings.

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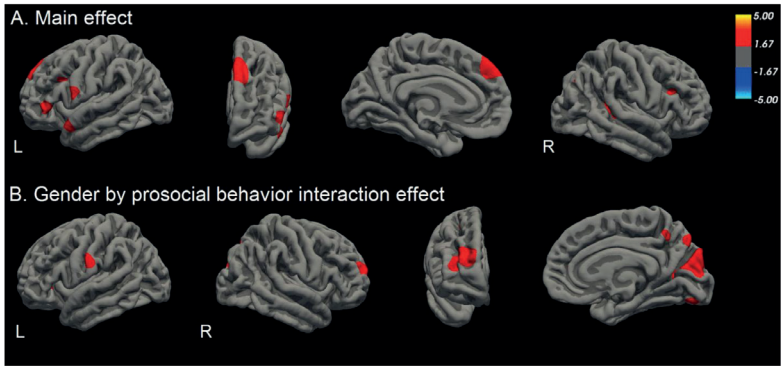

Supplementary Figure 3.1 | Averaged results of the bootstrapped association between cortical thickness and prosocial behavior. All clusters found in primary analysis are confirmed. Colors represent –log10 *p-value*.

Chapter 4

The honest truth about lying: demographic, cognitive and neural correlates of typical and persistent lie-telling

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ABSTRACT

This study examined situational, psychological and neurobiological factors associated with lie-telling in 7-to-10 year old children. By assessing lie-telling in a low and high lie-detectability condition, we differentiated between typical lie-telling and persistent lie-telling, and assessed the correlates of dishonesty in a sample of 163 children. Persistent lie-tellers could be discriminated from other children based on gender (more boys), lower age, lower IQ, less effortful control, and lower educated mothers. Compared to honest children and persistent lie-tellers, typical lie-tellers were more likely to be girls and to come from families with higher income. Compared to typical lie-tellers and honest children, persistent lie-tellers showed decreased activation in the bilateral ACC and right frontal pole during the low lie-detectability condition, suggesting decreased engagement in conflict monitoring and error detection. We did not find significant differences between honest children and typical lie-tellers.

INTRODUCTION

While honesty is considered fundamental to social relationships and societies, sometimes telling a lie may be more advantageous for the individual than speaking the truth. Several studies have shown that, given the opportunity, children from the age of three years onwards will lie to avoid punishment or to receive personal reward (Peskin, 1992; Talwar & Lee, 2008). Although parents generally discourage lie-telling, early lying may be a normative aspect of development (Talwar & Crossman, 2011). Problems arise when lying becomes habitual and inappropriately damages the interests of others, or when lie-telling compromises social relationships. The present study aimed to differentiate between honest children, children who showed typical lie-telling behavior and children who lied more persistently, and examined what demographic, cognitive, social and neurobiological factors are associated with lie-telling behavior.

In children, lie-telling has usually been assessed with the resistance-to-temptation paradigm (Lewis, Stanger, & Sullivan, 1989). In this paradigm, children are given the opportunity to commit a transgression. For example, they are told not to peek at a concealed object (e.g. a toy) while left alone in a room. Upon return, the experimenter asks the children if they have looked at the object. Talwar and Lee (2008) showed that 82% of 3-to-8 year old children peeked at the object during the experimenter absence. Moreover, 64% of those who peeked falsely denied the peeking. A study in 8-to-16 year old children showed that 84% of the children who peeked at the object were dishonest about the peeking (Evans & Lee, 2011). When assessing lie-telling for personal rewards, Peskin (1992) found that 87% of 5 year old children lied to a puppet about the location of a prize in order to keep the prize to themselves.

Since most children tell lies, lying may be an aspect of normative development. Indeed, the development of lie-telling seems to reflect children's emerging cognitive maturation (Talwar & Crossman, 2011). More specifically, lie-telling has been related to the development of theory of mind (ToM) and executive functions such as inhibitory control and working memory. In order to lie, children must understand that their mental state is not evident to others. To lie successfully, they must be capable of inhibiting the information they are trying to withhold while keeping the content of their lies in memory. Several studies have shown that the ability to falsely deny the occurrence of an event is related to inhibitory control and first-order belief understanding (the ability to understand another person's mental state), while more complex forms of lying are related to working memory and second-order belief understanding (the ability to understand another person's mental state about the mental state of someone else) (K. Lee, 2013; Talwar, Gordon, & Lee, 2007; Talwar & Lee, 2008).

Although a number of studies have examined the typical development of lie-telling, less is known about children that show persisting levels of dishonest behavior. However, as persistent dishonesty may pose problems for children themselves as well as their

environment, examining persistent lie-telling may provide important insights into the risk of child antisocial behavior. In Study 1, we examined what demographic, cognitive and social factors can differentiate between honest children, typical lie-tellers and persistent lie-tellers. In Study 2, we assessed the neurobiological correlates of (dis)honest behavior and explored whether the brain is differentially activated during lie-telling in typical lie-tellers and children who lie more persistently.

STUDY 1: PREDICTORS OF DISHONESTY

Studies using parent-reports on child dishonesty suggest that children coming from lower socio-economic status (SES) families and children with lower intelligence may be at increased risk for dishonest behavior (Cole & Mitchell, 1998; Stouthamer-Loeber & Loeber, 1986). Furthermore, children who show high rates of externalizing behavior may be at a higher risk for deceptive behavior. Indeed, fighting and relational aggressive behavior have been associated with increased dishonest behavior (Ostrov, Ries, Stauffacher, Godleski, & Mullins, 2008; Stouthamer-Loeber & Loeber, 1986). Also children with decreased self-regulatory control or less developed conscience may lie more frequently than others, as they focus on the short-term benefits of dishonesty and may not realize or care about the long-term consequences of their behavior (Talwar & Crossman, 2011). A study on cheating in children showed that severe cheating (but not typical cheating behavior) is related to lower levels of self-control and decreased internalization of rules of conduct (Callender, Olson, Kerr, & Sameroff, 2010). However, it is not clear whether these results are also applicable to lie-telling.

To differentiate between honesty, typical lie-telling as well as persistent lie-telling, the present study examined lying in a low lie-detectability and a high lie-detectability condition. In both conditions, behaving dishonestly would increase monetary gain. In the high lie-detectability condition, however, the children were made to believe that the experimenter could see whether they were being honest. In this condition, the children would have to weigh the monetary benefits of lying against the negative consequences of getting caught. Children who were dishonest in the low lie-detectability condition only were referred to as typical lie-tellers. Children who lied in both the low lie-detectability and the high lie-detectability condition were considered persistent lie-tellers.

Based on previous literature, we expected that the majority of the children will lie to increase their gain in the low lie-detectability condition. However, as the risk of getting caught increases, we expected that fewer children would behave dishonestly. As some lie-telling may be a typical aspect of development, we expected that honest children and typical lie-tellers would be characterized by similar demographic, cognitive and social factors. However, based on previous studies we expected that persistent lie-tellers would

be more likely to be from lower SES families, display higher levels of aggressive behavior, have a lower IQ score, and have less self-regulatory control and less developed conscience than honest children or typical lie-tellers.

METHODS

Participants

The present study was embedded within the Generation R Study, a prospective cohortfrom fetal life onwards in Rotterdam, the Netherlands (Jaddoe et al., 2012; Tiemeier et al., 2012). The honesty task was administered as part of a study of antisocial and prosocial behavior within the Generation R Study. For this purpose, we selected groups of highly aggressive children, highly prosocial children, and controls, based on parent-reported aggressive behavior (Child Behavior Checklist, administered at age 1.5, 3 and 6 years) (Achenbach & Rescorla, 2000) and prosocial behavior (Strengths and Difficulties Questionnaire, administered at age 6 years) (Goodman, 1997). In the group of children from Dutch origin who had Aggressive Behavior scores available at two or more time points, we distinguished three trajectories of aggression: a high, rising trajectory, an intermediate trajectory and a low trajectory (Wildeboer et al., 2015). Children in the high, rising trajectory were eligible for participation in the highly aggressive group. Children from the low aggression trajectory who had a minimum score of 14 on the SDQ Prosocial scale (range 5 – 15) were eligible for participation in the prosocial group. Children in the low aggression trajectory with a prosocial score <14 as well as children in the middle trajectory of aggression were eligible for participation in the control group. Supplementary Figure 4.2 summarizes recruitment and enrollment procedures.

Honesty task data were available for 174 children of the 291 invited children. Non-response analyses showed no differences between the invited children and the included children in gender, IQ, maternal education and family income. Nine children did not perform the high-risk version of the task because of time constrains. As we expected that children who were honest in the low lie-detectability condition would remain honest in the high lie-detectability condition, results on the high lie-detectability condition of the honesty task could be reliably imputed for one low lie-detectability honest child. Three children were excluded from the study as they had too many trials with missing responses on the honesty task. Therefore, analyses on the predictors of dishonesty were performed on 163 children.

Measures

Honesty task

The honesty task is a child-friendly adaptation based on Greene and Paxton (2009). During the task, participants were asked to predict a random computerized event. The participating

children were presented with computer images of two dogs. In the experimental condition, participants were requested to predict which dog (left or right) would receive a bone. Two seconds after the dogs were presented on the screen, the bone appeared above one of the dogs. Participants did not have to report their prediction. Instead, we only asked the children to report on their own accuracy after each trial. By pushing a button with middle finger of their dominant hand the children indicated that their response was correct, while a false response was indicated by pushing a button with the index finger. Each correct prediction was rewarded with €0.05. In the control condition, we asked the children to report whether one of the two dogs was wearing a collar. For the control task each correct answer was also rewarded with €0,05. The children received their monetary reward at the end of the scanning session.

Children were asked to perform the task first in a low lie-detectability condition and then in a high lie-detectability condition. In the high lie-detectability condition, the experimenter told the children that (s)he was able to evaluate whether or not the children were being honest. As the children performed the task in a MRI scanner, they were led to believe that their honesty could indeed be evaluated. During a debriefing at the end of the imaging session, the children were told that the experimenter was in fact unable to see whether or not they had been honest.

We used images of six different dogs. For each dog, we created one image with a collar and one image without a collar. The task consisted of 36 trials, presented in 12 blocks of three trials each (6 experimental blocks and 6 control blocks). The allocation of the bone was at random. We created four pseudo-random versions of the order of the dogs, two of which started with an experimental block, and two with a control block. Each child was randomly assigned to one of the four versions. For details on stimulus presentation, see Figure 4.1.

Children of whom more than 25% of the trails on the experimental task were missing data were excluded from the analyses (n=1 in the low lie-detectability condition, n=2 in the high lie-detectability condition). Participants who reported improbably high levels of accuracy (one-tailed binomial test, p<.05; more than 13 correct guesses (72%) in 18 trials) were classified as dishonest. All other participants were classified as honest. Based on the two conditions of the task, children were classified as (a) honest, (b) typical lie-tellers, or (c) persistent lie-tellers. For the study on the predictors of dishonesty (Study 1), only data on the experimental trials (and not the control trials) were analyzed.

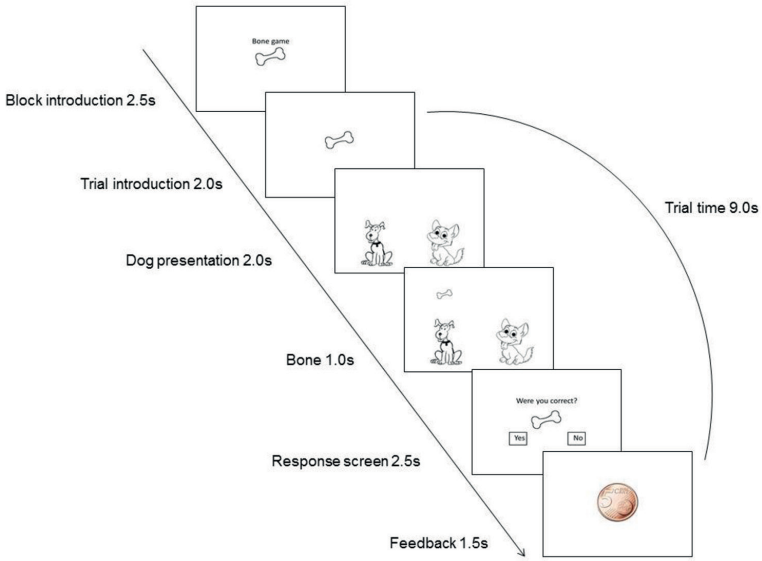

Figure 4.1 | Stimulus presentation honesty task

Intelligence

Intelligence was assessed using two subtests (Mosaics – visuospatial abilities, and Categories – abstract reasoning) of the non-verbal intelligence test Snijders-Oomen Niet-verbale intelligentie Test-Revisie (SON-R 2 ½ – 7) when the children were on average 6 years of age (Tellegen, Winkel, Wijnberg-Williams, & Laros, 2005). The raw test scores were standardized into nonverbal IQ scores.

Effortful control

To assess self-regulatory control, we used the Effortful Control scale of the Children's Behavior Questionnaire – Very Short Form (CBQ-VSF), a parent-report measure of temperament for children from 3 to 8 years of age (Putnam & Rothbart, 2006). The CBQ-VSF was filled out by the primary caregiver when the children were on average 6 years of age. The Effortful Control scale consists of 12 items such as "My child is very focused when he/she is drawing or coloring" that were rated on a 7-point Likert scale ranging from 'totally incorrect' to 'very correct' (*M*=5.30, *SD*=0.64). Internal consistency of the Effortful Control scale was acceptable, α=0.68.

Conscience development

The My Child Questionnaire is a parent-reported questionnaire on conscience development (Kochanska, DeVet, Goldman, Murray, & Putnam, 1994). The My Child Questionnaire was abbreviated with consent from Kochanska (personal communication) and was completed by the primary caregiver when the children were on average 6 years old. For the present study we used the abbreviated Guilt scale (8 items, e.g. "My child cries easily when spoken to about something naughty that he/she has done", α=.70) and the Internalized Conduct scale (6 items, e.g. "My child rarely repeats behavior that was previously forbidden, not even in the absence of adults", α=.73). Items were scored on a 7-point Likert scale ranging from 'not applicable at all' to 'fully applicable' (*M*=4.10, *SD*=0.90 for Guilt, *M*=4.76, *SD*=0.90 for Internalized Conduct).

Demographic information

Information on maternal education and family income was obtained using a questionnaire when the children were on average 6 years old. Maternal education level was assessed as the highest completed education and was categorized into secondary (lower and intermediate vocational training), and higher education (higher vocational education and university).

Information on family net income was obtained using 11 categories ranging from less than 800-to-5600 or more per month. When information on family income was missing (*n*=15), the median was imputed. Family income scores were square root transformed to approximate a normal distribution.

Statistical analyses

Chi-square analyses and *t*-tests were performed to assess the relation between background variables (gender, age, trajectory group, version of task) and honesty for the low lie-detectability and high lie-detectability condition separately. The correlations among the predictor variables were assessed using Pearson's correlation coefficient in the case of two continuous variables, point-biserial correlations in the case of one continuous and one dichotomous variable, and Phi-coefficients for correlations between two dichotomous variables. We performed a discriminant analysis to assess the difference between honest children, typical lie-tellers and persistent lie-tellers. For children with missing data on two or less of the predictors (*n*=16), missing data were imputed using linear regression analysis. For children with missing data on three or more predictors (*n*=14), the discriminant analysis was performed with and without mean imputation. Moreover, analyses were performed both including and excluding children who did not perform above chance level on the control task (*n*=17) as these children may have had problems understanding the task. As we aimed to predict membership to three groups, two discriminant functions were calculated.

RESULTS

Table 4.1 shows the demographic characteristics of the sample. Forty-two children (26%) were classified as honest in the low lie-detectability condition. In the high lie-detectability condition 107 children (66%) were honest (χ^2=21.393, p<.001, φc=.36). The version of the task (i.e. order of experimental and control blocks) did not predict honesty (χ^2=2.75, p=.43, and χ^2=0.46, p=.93 for low and high lie-detectability respectively). Therefore, version was not included in the discriminant analysis. The correlations among the predictor variables can be found in Table 4.2.

Table 4.1 | Sample Characteristics

	n	Total group	Honest (*n*=40)	Typical lie-tellers (*n*=69)	Persistent lie-tellers (*n*=54)	*p*
Categorical						
Girl	163	82 (50.3)	20 (50.0)[ab]	44 (63.8)[a]	18 (33.3)[b]	.003
Trajectory group	163					
Prosocial		49 (30.1)	10 (25.0)[a]	33 (47.8)[a]	17 (31.5)[a]	
Aggressive		57 (35.0)	13 (32.5)[a]	23 (33.3)[a]	21 (38.9)[a]	.76
Control		57 (35.0)	17 (42.5)[a]	24 (34.8)[a]	16 (29.6)[a]	
Maternal higher education	163	125 (76.7)	36 (90.0)[a]	53 (76.8)[ab]	36 (66.7)[a]	.02
Continuous						
Family income	158	8.51 (2.09)	8.03 (2.42)[a]	8.97 (1.79)[b]	8.27 (2.11)[ab]	.08
Age	163	8.69 (0.77)	9.00 (0.83)[a]	8.65 (0.76)[b]	8.42 (0.63)[b]	.001
IQ	150	105.83 (13.74)	108.82 (10.92)[a]	107.57 (12.76)[a]	101.70 (14.65)[b]	.01
Effortful control	147	5.32 (0.63)	5.43 (0.57)[a]	5.43 (0.56)[a]	5.07 (0.70)[b]	.004
Guilt	147	4.10 (0.91)	4.24 (0.89)[a]	4.11 (0.88)[a]	3.99 (0.98)[a]	.45
Internalized conduct	148	4.80 (0.89)	4.96 (0.88)[a]	4.81 (0.84)[a]	4.68 (0.95)[a]	.38

Note. In case of categorical variables, numbers represent n(%). Significance was tested using logistic regression analyses. In case of continuous variables, numbers represent M(SD).
Significance was examined using ANOVA analyses. Values that do not share the same subscript are significantly different (p<.05).

Table 4.2 | Correlations Between Predictor Variables

	Age	Gender	Maternal education	Family income	IQ	Guilt	Internalized conduct
Gender (0 = boy; 1 = girl)	-.08						
Maternal higher education	.01	-.08					
Family income	-.02	.12	.30***				
IQ	-.06	.00	.20*	.03			
Guilt	.09	.10	.02	-.08	.15		
Internalized conduct	-.10	.06	.07	-.08	.16	.21*	
Effortful control	-.02	.22**	.13	.05	.12	.19*	.30***

Note. * $p<.05$, ** $p<.01$, *** $p<.001$

Predictors of honesty, typical lie-telling, and persistent lie-telling

Forty children (25%) were classified as honest in both conditions, 69 children (42%) were classified as typical lie-tellers and 54 children (33%) were classified as persistent lie-tellers. Group characteristics can be found in Table 4.1.

We used a discriminant analysis to predict honesty group from gender, age, maternal higher education, family income, IQ, Effortful Control, Guilt, and Internalized Conduct. Trajectory group, Internalized Conduct and Guilt were excluded from the model, as their factor loadings were smaller than .30 for both discriminant functions.. The two discriminant functions had a combined $\chi^2(10)=54.89$, $p<.01$. After removal of the first function, the second discriminant function was significant, $\chi^2(5)=14.19$, $p<.05$. The canonical r^2 of the first and second discriminant function were 0.25 and 0.09, respectively. As shown in Figure 4.2, the first function separates persistent lie-tellers (M=-0.72, SD=1.15) from typical lie-tellers (M=0.23. SD=0.94, $O.R.$=2.66, $p<.001$) and honest children (M=0.63, SD=0.91, $O.R.$=4.22, $p<.001$), but did not separate typical lie-tellers from honest children ($O.R.$=1.59, p=.08). The second function separated the typical lie-tellers (M=0.34, SD=0.87) from the honest children (M=-0.35, SD=1.20, $O.R.$=1.95, p=.004) and persistent lie-tellers (M=-0.10, SD=0.90, $O.R.$=0.60, p=.03), (*p-values* were Bonferroni corrected). Table 4.3 shows the function loadings of the predictors on the two discriminant functions. Persistent lie-tellers could be discriminated from typical lie-tellers and honest children by lower age, lower intelligence, less self-regulatory control, lower maternal education, and were more likely to be boys. Typical lie-tellers could be discriminated from honest children and persistent lie-tellers based on a higher family income, and were more likely to be girls. Thus, whereas persistent lie-tellers could be discriminated from the other children based on demographic and cognitive variables, typical lie-tellers were highly similar to honest children and differed only on family income and gender.

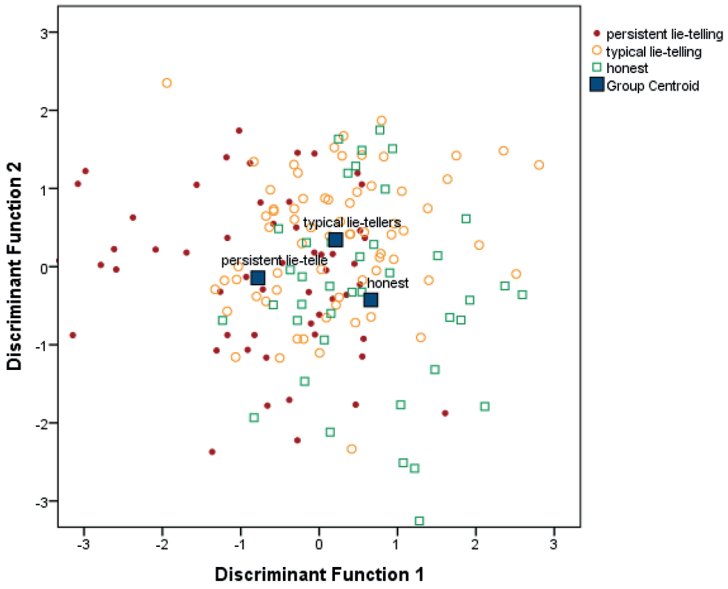

Figure 4.2 | Group centroids on the two discriminant functions

Table 4.3 | Correlation Between Predictors and Discriminant Functions

	Function 1	Function 2
Age child honesty task	.51	-.29
Effortful control	.46	.27
IQ	.43	.02
Maternal higher education	.37	-.25
Family income	.07	.65
Gender	.36	.58

Results were identical when analyses were performed with and without mean substitution for missing data, and they were also similar when children who did not perform above chance level on the control task were excluded.

STUDY 2: THE NEUROBIOLOGICAL CORRELATES OF TYPICAL AND PERSISTENT LIE-TELLING

Functional MRI studies may have an important function in the search for insights into the processes related to lie-telling. Neuroimaging studies suggest involvement of the prefrontal cortex (e.g. dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC) and anterior prefrontal cortex (APFC, or frontal pole)) and anterior cingulate cortex (ACC)

during dishonest behavior (Abe, 2011; Christ, Essen, Watson, Brubaker, & McDermott, 2009; Greene & Paxton, 2009; Ito et al., 2011; Langleben et al., 2002; T. M. C. Lee, Lee, Raine, & Chan, 2010; Spence et al., 2001). Activation in these regions has been implicated in cognitive processes related to deception, such as working memory, inhibitory control, and error detection (Abe, 2011). Also the amygdala, a region known for its involvement in the processing of emotional information, has been implicated in dishonest behavior. As the imaging research on honesty has mostly focused on adult samples, little is known about brain correlates of dishonesty in children. The frontal lobes continue to mature even post-adolescence (Gogtay et al., 2004). Therefore, dishonesty in children may rely on spatially distinct, or alternatively morphologically different, regions compared to adults. To our knowledge, only one neuroimaging study has examined lie-telling in children (Yokota et al., 2013). Instead of associations with the prefrontal cortex, results of this study suggest a role for the inferior parietal lobule in deception in children.

Even in the adult literature, not much is known about neural activation underlying typical versus persistent lie-telling. Structural imaging studies have found increased prefrontal white matter in pathological liars, especially in orbitofrontal, middle and inferior frontal gyri (Yang et al., 2007). Conversely, individuals suffering from lesions in the orbitofrontal cortex (OFC) are sometimes reported to be pathologically truthful (Spence & Kaylor-Hughes, 2008). A functional MRI study on lie-telling in individuals suffering from antisocial personality disorder has shown that frontal brain activation during deception is negatively correlated with the capacity to lie (Jiang et al., 2013). However, it is unclear whether these results hold for healthy individuals or children.

Based on these findings, we hypothesized that children who lied would show increased prefrontal activation in comparison to children who performed the task honestly. Moreover, as lie-telling may require a greater cognitive effort for typical lie-tellers compared to persistent lie-tellers, we expected that typical lie-teller would show a larger increase in prefrontal activity than children who lie more persistently.

METHODS

Participants

Honesty task data were available for 174 children of the 291 invited children. As in Study 1, three children were excluded because they had too many trials with missing data (1.7%). For the imaging analyses, all children without data on the high lie-detectability condition were excluded (n=9). An additional four children were excluded due to technical problems (2.3%). Forty-nine children (28.2%) were excluded due to excessive motion (>0.5mm mean relative displacement). Finally, as brain activation in response to honesty trials was contrasted against brain activation in response to control trials, 10 children (5.7%) were excluded

because their score on the control task was not above chance level. As a consequence, fMRI analyses were performed on 99 children (54 girls) of whom 28 children were honest (14 girls), 43 were typical lie-tellers (28 girls) and 28 were persistent lie-tellers (12 girls).

Non-response analyses showed that the excluded children did not differ in (dis)honesty group, gender, effortful control, family income, and maternal education. However, excluded children were younger than included children, $t(167)=-3.17$, $p=.002$, and had lower IQ scores, $t(171)=-2.53$, $p=.01$.

Measures

Honesty task

The honesty task is described in detail above for Study 1. In brief, the participating children were presented with computer images of two dogs. In the experimental condition, participants were requested to predict which dog (left or right) would receive a bone. Participants did not have to report their prediction. Instead, we only asked the children to report on their own accuracy after each trial. By pushing a button with middle finger of their dominant hand the children indicated that their response was correct, while a false response was indicated by pushing a button with the index finger. Each correct prediction was rewarded with €0.05. To tease apart the BOLD signal associated with (dis)honesty, we implemented a control task which was equal to the experimental task in visual characteristics and working memory load, but did not provide an opportunity for dishonest gain. Instead of asking to report on the accuracy of their prediction, in the control condition, the children were asked to report whether one of the two dogs was wearing a collar. For the control task each correct answer was also rewarded with €0,05. The children received their monetary reward at the end of the scanning session.

The task was coded in PsychoPy version 1.7 (Peirce, 2007), and was administered using a Windows PC. A projector outside of the MRI suite was used to display the task on a large screen located at the front of the MRI system, which was viewable through a mirror mounted on the top of the head coil. Prior to beginning the task, a test-screen was displayed in order to ensure the children could see the entire viewable area of the task, and a short practice version was administered while the children were in the scanner to ensure they remembered how to complete the task, and to confirm the equipment was functioning normally. All responses were registered using a fiber optic response box (Current Designs, Philadelphia, PA, USA).

Imaging data

A description of the neuroimaging component of the Generation R Study has been provided elsewhere(White et al., 2013). Briefly, children were first familiarized with a mock scanning session. MRI scanning was performed on a GE Discovery MR 750 3 T scanner (General Electric, Milwaukee, WI, USA). T1-weighted inversion recovery fast spoiled

gradient recalled (IR-FSPGR) sequence was obtained using an 8 channel head coil with the following parameters: TR=10.3 ms, TE=4.2 ms, TI=350 ms, NEX=1, flip angle=16°, readout bandwidth=20.8 kHz, matrix 256x256, imaging acceleration factor 2, and an isotropic resolution of 0.9x0.9x0.9 mm^3. The fMRI-task utilized a gradient-echo blood oxygen level dependent (BOLD) EPI sequence with a TR=2,000 ms, TE=30 ms, flip angle=90°, matrix 64x64 and voxel resolution of 3.6x3.6x4.0 mm^3. The duration of the fMRI paradigm is 6 min. 24 s. (192 TRs).

Statistical analyses

Group differences in reaction times were performed using ANOVA analyses. Preprocessing and statistical analysis of the imaging data were performed using FSL (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; Smith et al., 2004). Brain extraction was performed via BET and motion correction was performed using MCFLIRT. Spatial smoothing was applied with a Gaussian kernel of 5 mm (FWHM). Functional MRI data from each child were spatially normalized to their own high resolution T1 image (boundary-based registration (BBR, Greve & Fischl, 2009), 90 degree search) and then to our own child brain template (12 degrees of freedom (DOF), 90 degree search) using FSL's FLIRT registration tool. Given the young age of the sample, it was important to use an age-appropriate template for registration of the functional data to standard space. To construct the age-appropriate template, an iterative, nonlinear approach (Sanchez, Richards, & Almli, 2012) was applied to 130 T1-weighted images (rated as having excellent quality). More information on the construction of the template is provided in Supplementary Text 4.1. For six children, registration to the high-resolution T1 image using BBR failed. For these children, functional imaging data were registered to the high resolution T1 image using an affine transformation, 90 degree search. After preprocessing, statistical analyses were performed at the single-subject level using the general linear model within FSL (FEAT). A design matrix was created by convolving the blocks with a gamma hemodynamic response function. Both standard motion parameters and additional motion confound EVs as obtained from fsl_motion_outliers (DVARS, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers) were added to the regression model to address common problems resulting from motion (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). The task consisted of 36 trials, presented in 12 blocks of three trials each (6 experimental blocks and 6 control blocks). For the GLM, we contrasted experimental vs. control blocks (with each block starting at the instruction of the first trial, and finishing after the feedback screen of the third and final trial).

For the low lie-detectability condition, mixed-effect group analyses were performed using FSL's FLAME I + II (FMRIB's local analysis of mixed effects) module. Age, gender, IQ, trajectory group, and version of the task (i.e., pseudo-randomized order) were used as covariates. Mean group contrasts were created. Next, honest children were contrasted against typical lie-tellers and persistent lie-tellers. Then, a contrast was created to compare

typical lie-tellers vs. persistent lie-tellers in the low lie-detectability condition. Although these groups cannot be discriminated based on their behavior on the low lie-detectability condition, results from the high lie-detectability condition suggest that typical lie-tellers and persistent lie-tellers form distinct groups and thus may show differential brain activation patterns related to dishonesty in the low lie-detectability condition. Statistical maps were thresholded using clusters determined by $Z>2.3$ and a (corrected) cluster significance threshold of $p<0.05$. Due to the number of comparisons performed, we only report findings with *p-value*<.008.

We repeated the fMRI analyses comparing the trajectory groups (controlling for age, gender, IQ, lie-telling group, and version of the task) to rule out the possibility that effects of lie-telling can be explained by our sampling strategy.

We chose not to examine group differences in the high lie-detectability condition or within-subject differences in low lie-detectability versus high lie-detectability lie-telling, as the focus of the present study is on the neural correlates of lie-telling and not on the effects of perceived lie-detectability.

RESULTS

There were no significant differences in reaction times between honest children, typical lie-tellers and persistent lie-tellers in the low lie-detectability condition, $F(2,98)=0.70$, $p=.93$.

To examine the neural correlates of lying, we compared honest children with typical lie-tellers and persistent lie-tellers, and typical lie-tellers with persistent lie-tellers on the experimental blocks versus control blocks contrast. Group mean effects can be found in Supplementary Table 4.1 and Supplementary Figure 4.3. For all groups, the experimental task was associated with increased activation in regions of the default mode network (ACC, bilateral angular gyrus) compared to the control task. Significant group differences in the experimental vs. control contrast can be found in Table 4.4 and Figure 4.3. We did not find any significant differences between honest children and typical lie-tellers. Compared to persistent lie-tellers, honest children showed increased activity in the bilateral ACC, extending to the right frontal pole ($p<.001$, Figure 4.3a). The reversed contrast (persistent lie-tellers > honest children) provided no significant results. Although typical lie-tellers and persistent lie-tellers were undistinguishable based on their behavior during the low lie-detectability condition, typical lie-tellers showed more activity in a cluster including the bilateral ACC, extending to the right frontal pole ($p<.001$, Figure 4.3b) compared to persistent lie-tellers in the low lie-detectability condition. The reversed contrast (persistent lie-tellers > typical lie-tellers) provided no significant results.

We did not find any differences in BOLD signal between the highly aggressive, highly prosocial, and control groups during the low lie-detectability condition.

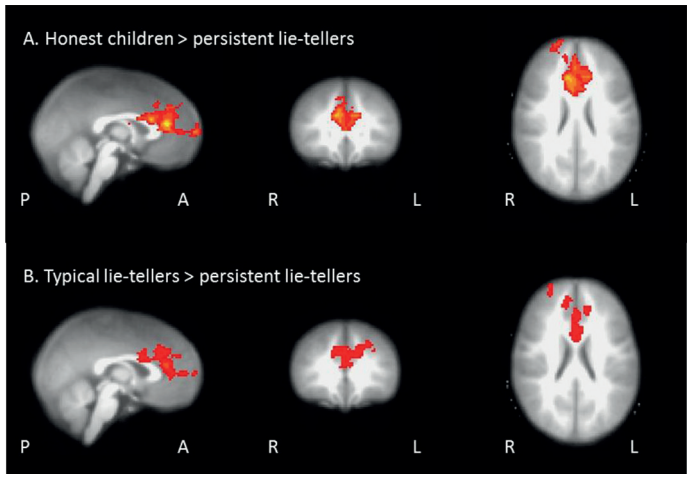

Figure 4.3 | Group differences of low lie-detectability lie-telling (experimental blocks – control blocks). **A.** Compared to persistent lie-tellers, honest children showed increased activity in the bilateral anterior cingulate cortex and right frontal pole. **B.** Compared to persistent lie-tellers, typical lie-tellers showed increased activity in the bilateral anterior cingulate cortex and right frontal pole.

Table 4.4 | Group Differences in the Experimental Blocks Versus Control Blocks Contrast

Cluster number	Region	Cluster size	MNI coordinates (mm)			*z*	*p* (corr.)
			x	y	z		
	Honest children > typical lie-tellers: n.s.						
	Typical lie-tellers > honest children: n.s.						
	Honest children > persistent lie-tellers						
1	R ACC	2528	2	36	16	4.75	<.001
	R ACC		5	13	21	4.00	
	R ACC		9	25	25	3.98	
	L ACC		-4	19	19	3.93	
	R ACC		2	21	21	3.86	
	R frontal pole		2	66	8	3.85	
	Persistent > honest children: .n.s.						
	Typical lie-tellers > persistent lie-tellers						
1	ACC	1711	2	34	16	4.36	<.001
	R paracingulate		11	46	28	4.03	
	R frontal pole		11	44	32	3.64	
	R frontal pole		22	48	34	3.57	
	L WM/ACC		-2	6	23	3.56	
	L WM/ACC		2	10	23	3.51	
	Persistent lie-tellers > typical lie-tellers: n.s.						

Note. ACC = anterior cingulate cortex; WM = white matter

GENERAL DISCUSSION

The present study examined lie-telling in a low lie-detectability and a high lie-detectability condition. In the low lie-detectability condition, more than three quarters of the children were dishonest. When the children believed their honesty could be evaluated, only one third of the children lied. In their paper on situational morality, Van IJzendoorn et al. (2010) suggest that morality may not be predicted by genes, attachment, temperament or parenting, but instead, situational demands may be the best predictor of moral behavior. In the present study several children showed dishonest behavior only in the low lie-detectability condition. Thus, we show that situational demands are also an important predictor of deception.

While many typical lie-tellers refrained from lying in the high lie-detectability condition, some children continued to behave dishonestly even when they believed their honesty was being evaluated. Compared to typical lie-tellers and honest children, these persistent lie-tellers are younger, have lower educated mothers, and are more likely to be boys. These differences in lie-telling may partly be explained by socialization processes by parents, teachers and peers. Socialization may cause older children to conform to moral rules and standards and thus lie less frequently than younger children (Talwar & Crossman, 2011). Lower educated mothers may adopt parenting styles that are suboptimal for moral development (McLoyd, 1998), and teachers may have lower expectations of children from lower educated parents (Alexander, Entwisle, & Thompson, 1987). Moreover, parents and teachers generally expect girls to be more social than boys (Mills & Rubin, 1990), and may enforce moral rules on girls more strongly than on boys. Additionally, compared to honest children and typical lie-tellers, persistent lie-tellers were characterized by lower cognitive abilities (lower IQ and less self-regulatory control). According to theories by Kohlberg and Bandura, moral development is highly depended upon cognitive abilities (Bandura, 1991; Kohlberg, 1975) and the development of lie-telling has been persistently related to cognitive maturation (Talwar et al., 2007; Talwar & Lee, 2008). In 8-to-12 year old children, higher cognitive abilities are related to more honest behavior but also to more sophisticated dishonest tactics (Ding et al., 2014). Our results correspond well with these findings, as they show that both honest children and children who show more typical dishonest behavior have higher IQ scores and more self-regulatory control compared to persistent lie-tellers.

Children who were dishonest in the low lie-detectability condition but refrained from dishonest behavior in the high lie-detectability condition were characterized by higher family income than consistently honest children or persistent lie-tellers. Although their behavior does not conform with moral rules, by lying in the low lie-detectability condition only, the typical lie-tellers increased their monetary reward but avoided the adverse social consequences of getting caught while lying. Compared to honest children and persistent lie-tellers, typical lie-tellers were more likely to be girls. Whereas studies based on parent

or teacher reports of lie-telling have suggested that boys lie more frequently than girls (Gervais, Tremblay, Desmarais-Gervais, & Vitaro, 2000; Stouthamer-Loeber, 1986), studies using the resistance-to-temptation paradigm have suggested that boys and girls are equally likely to behave dishonestly (Callender et al., 2010; Talwar & Lee, 2008). These discrepant findings suggest that informants may be biased regarding lie-telling behavior of children. Alternatively, girls may be more skilled at lying and get caught less frequently when telling lies (thus parents report less lying). Our findings support this later notion, as we show that girls do lie when they believe their behavior is covert, but refrain from lying when they believe their honesty was evaluated. Moreover, in line with studies on aggression (Crick & Grotpeter, 1995), our results may point to a more covert nature of immoral behavior in girls, while boys' immoral behavior may be covert as well as overt.

The present study also assessed the neurobiological correlates of child dishonest behavior. Following the literature on adult samples (Abe, Suzuki, Mori, Itoh, & Fujii, 2007; Christ et al., 2009; Ito et al., 2011; Langleben et al., 2002), we expected to find increased PFC and ACC activation in dishonest children (typical lie-tellers or persistent lie-tellers) compared to honest children. Conversely, our fMRI results mimicked our findings presented in Study 1: we found similar neural activation patterns in honest children and typical lie-tellers. However, compared to both honest children and typical lie-tellers, persistent lie-tellers showed decreased activation in the bilateral ACC and right frontal pole. The ACC is involved in conflict monitoring and error detection (Carter et al., 1998), and also the frontal pole has been implicated in the monitoring of outcomes (Koechlin, 2011). Therefore, the present findings suggest that persistent lie-tellers may engage in less monitoring and experience less conflict during opportunities for lie-telling than honest children and typical lie-tellers. Below we offer several explanations.

Study 1 showed that persistent lie-tellers could be discriminated from typical lie-tellers and honest children by displaying less effortful control. As effortful control has been defined as 'the ability to inhibit a dominant response in order to perform a subdominant one, to detect errors, and to engage in planning', the decrease in ACC and frontal pole activation in persistent lie-tellers compared to honest children and typical lie-tellers may reflect their decreased tendency to monitor their performance. Alternatively, persistent lie-tellers may care less about their moral behavior and may thus experience less conflict compared to honest children and typical lie-tellers. It is also possible that persistent lie-tellers used a different strategy compared to the honest children and typical lie-tellers during the experimental task. The honest children and typical lie-tellers may have performed the task as instructed: they choose one of the two dogs and later checked the accuracy of their prediction. In case of a false prediction, the honest children then made an honest report on their accuracy, while the typical lie-tellers lied. The persistent lie-tellers may have failed to or decided not to pick one of the dogs and thus did not check the accuracy of their prediction, resulting in lower ACC activation compared to honest children and typical lie-tellers. When

the response screen appeared, they reported that they were correct without actually having made a prediction. However, we excluded children who performed poorly on the control task. For the control task, children were asked to report whether one of the dogs was wearing a collar. Thus, children needed to pay attention to the dogs in order to give the right answer. Therefore, we expect that all children also paid attention during the experimental trials. Finally, persistent lie-tellers may have misunderstood the task, but did realize that pressing the 'yes' button increased their monetary reward and thus were characterized as dishonest. However, we think that this explanation is unlikely. Although persistent lie-tellers had lower IQ scores compared to the two other groups, their mean IQ score was still 102. Moreover, we excluded children who performed poorly on the control task. In order to obtain good scores, persistent lie-tellers had to employ different strategies for the control and experimental task, as 'yes' is always the correct answer for experimental trials, while for control trials 'yes' or 'no' could both be the correct answer. As persistent dishonest children were able to switch between response strategies, it is unlikely that the experimental task was too difficult from them.

While fMRI studies in adults suggest an increased demand on executive functions in dishonest behavior, we did not find increased activation in typical or persistent lie-tellers in comparison with honest children. Our results, therefore, suggest that in children, lie-telling may be a more automatic process rather than a controlled one. However, studies on deception in children show that dishonesty is related to the development of executive functioning skills, such as inhibitory control and working memory (Evans & Lee, 2011; Talwar et al., 2007). This explanation, therefore, seems unlikely. Alternatively, this finding may be a consequence of the paradigm we employed. Dishonest children need not be dishonest in all trials, as approximately 50% of their predictions would be expected to be correct and would thus be honest wins. In order to increase the covert nature of dishonest behavior in the low lie-detectability condition, we did not ask the children to report their prediction and therefore we cannot tease apart honest from dishonest wins. As we used a blocked design, effects of dishonest behavior may have been diluted by the number of honest trials.

Although previous studies have found associations between conscience development and lie-telling (Shalvi & Leiser, 2013; Wolf, Cohen, Panter, & Insko, 2010), in the present study, conscience as well as prosocial and aggressive behavior were unrelated to (dis) honesty. As lie-telling in the present study was not harmful to other people, the absence of an association between lie-telling and reported conscience, aggression, and prosocial behavior may not be unexpected. Moreover, although moral behavior may be associated with affective processes, it has strong roots in cognitive development as well (Bandura, 1991; Talwar & Lee, 2008). The results of both Study 1 and Study 2 suggest that dishonesty in the present study involves cognitive processes, rather than affective processes, which may explain the lack of associations between dishonesty and aggression, prosocial behavior, and conscience. Alternatively, the absence of an association between conscience and (dis) honesty may be related to measuring conscience with brief questionnaires.

Some limitations should be noted. In order to increase the covert nature of dishonest behavior in the low lie-detectability condition, we did not ask the children to report their prediction. However, as a consequence, we are unable to guarantee that all children defined as honest were actually honest on all trials. Moreover, we did not ask the children whether they believed their honesty could be evaluated in the high lie-detectability condition. Therefore, it is possible that the children who were dishonest during the high lie-detectability condition continued to lie because they did not believe their honesty could be evaluated. However, in the high lie-detectability condition, children who refrained from lying had higher IQ scores compared to the children who continued to lie. Therefore, we expect that this effect is minimal.

CONCLUSION

Dishonest behavior in children seems largely dependent on the estimated likelihood of getting caught. A large majority of 7 – 10 year old children lie in low lie-detectability conditions, but a substantial number of these children refrain from lying when they feel monitored and run the risk of getting caught. Children who do lie in the high lie-detectability condition differ from honest or typical lie-tellers in displaying less developed executive functioning and lower general cognitive abilities. In a somewhat cynical way, dishonesty might be defined as a lack of cognitive resources to avoid getting caught in the act. The neurobiological data mimicked our behavioral results in showing similar neural patterns in honest children and typical lie-tellers, while persistent lie-tellers differed from typical lie-tellers and honest children in showing less brain activation of the anterior cingulate cortex and frontal pole during the task. These findings suggest that persistent lie-tellers engage in less conflict monitoring and error detection during lie-telling. Combined, the results of our study suggest that, compared to honest children and children who display typical lie-telling behavior, children who show persistent lie-telling behavior seem to form a distinct group with different cognitive and neurobiological characteristics.

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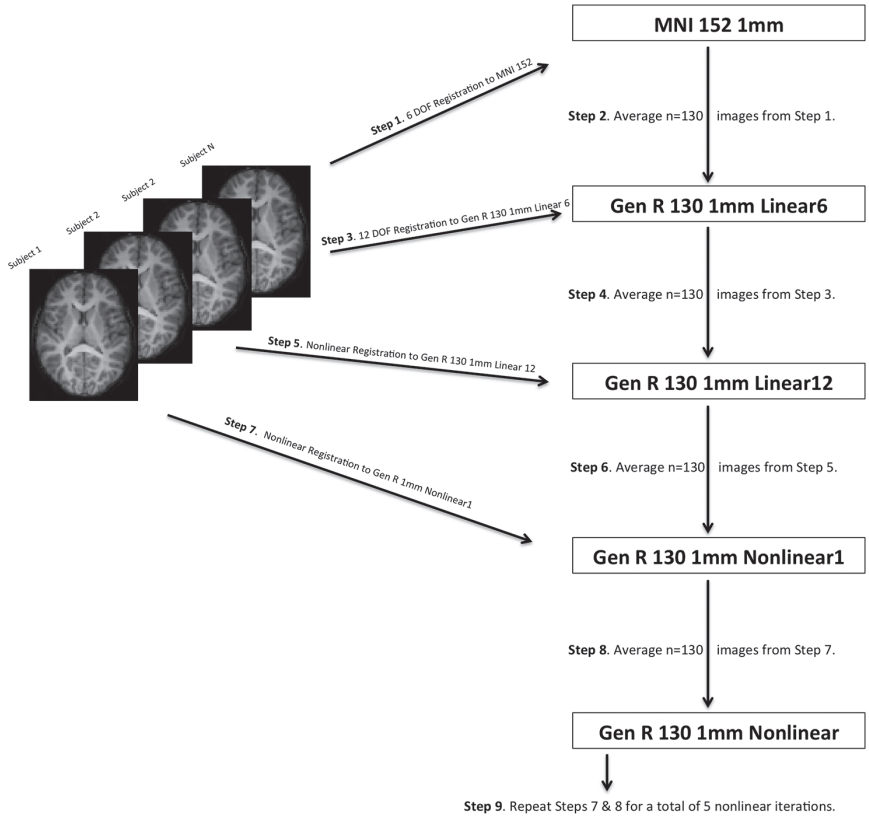
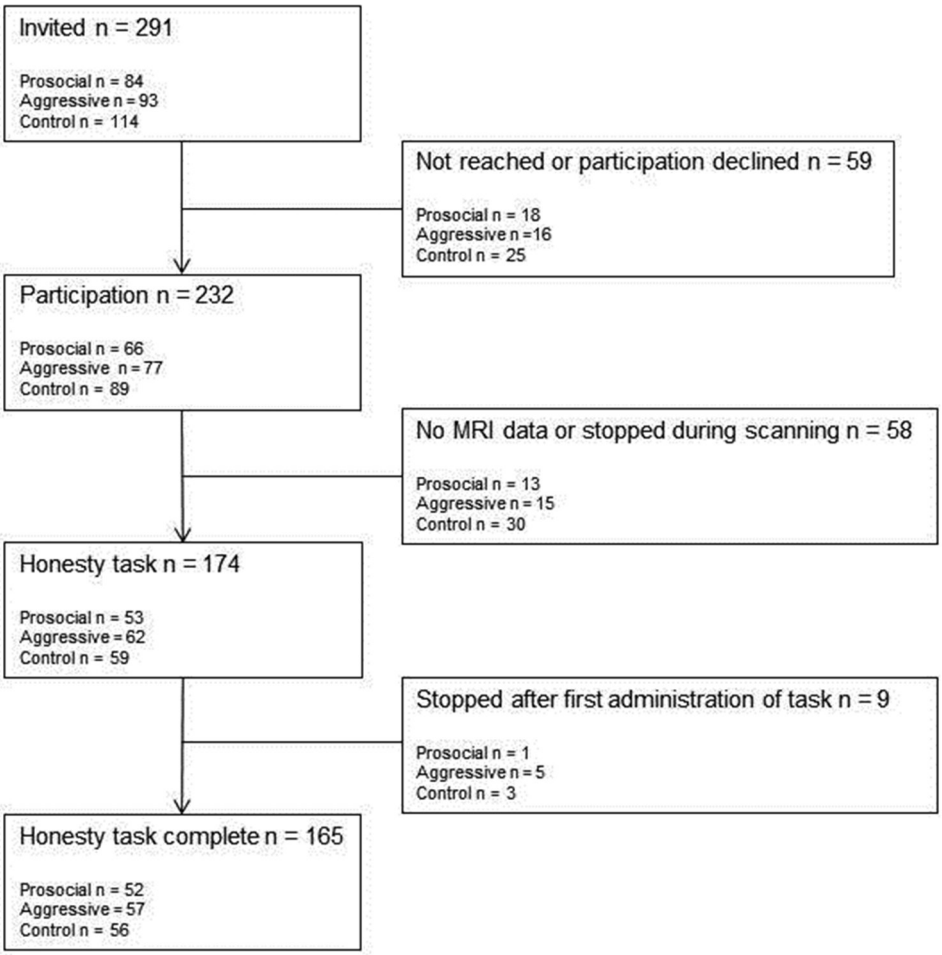
SUPPLEMENTARY TEXT 4.1

Study-specific, age-appropriate template for registration

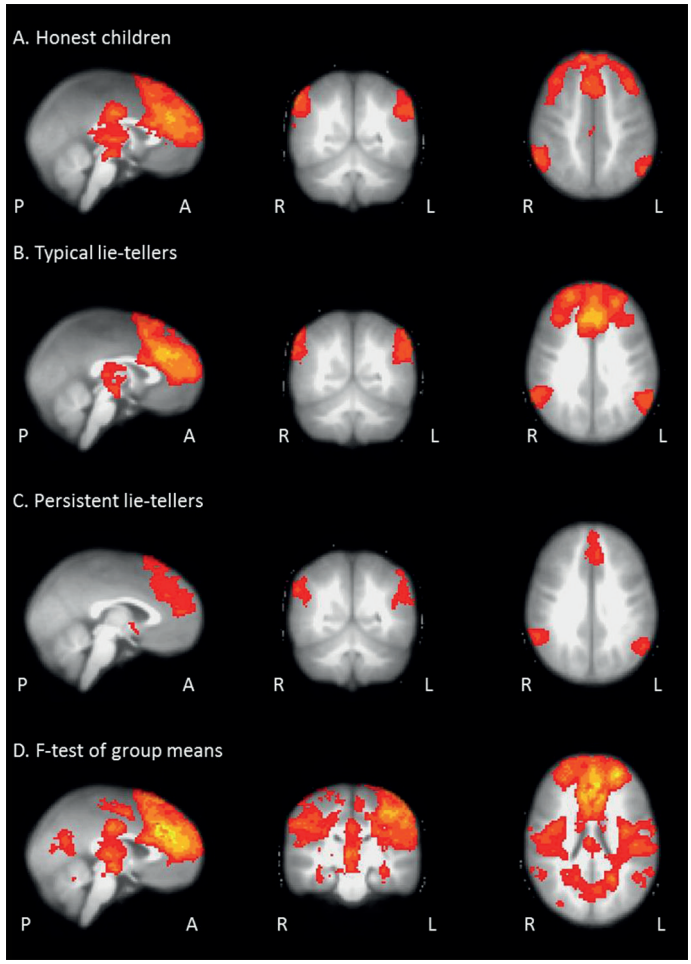
Given the age of the sample, it was important to use an age-appropriate template for registration of the functional data to standard space. One hundred thirty T1-weighted images from children without behavioral problems, also rated as having excellent quality, were used to construct the structural template for registration. An iterative approach using both linear and nonlinear algorithms was used (Sanchez et al., 2012), and is represented graphically in the supplemental data section (Supplementary Figure 4.1). Briefly, T1-weighted images from each of the 130 subjects were first aligned to the MNI-152 1mm brain using a linear, 6 degree of freedom approach (FLIRT). All registered images were then averaged and used as the template brain for the subsequent step, which was a nonlinear registration (FNIRT). Once again, the result from the nonlinear registration was averaged and used as the template for the subsequent iteration. This routine continued for a total of five nonlinear iterations, where it has been shown the template image stabilizes considerably (Sanchez et al., 2012). The result of the fifth and final nonlinear registration was averaged, resampled to 2mm isotropic resolution, and then used as the standard-space template for all FMRI datasets.



Supplementary Figure 4.1 | Study-specific, age-appropriate template for registration



Supplementary Figure 4.2 | Flowchart of participant inclusion



Supplementary Figure 4.3 | Group mean effects on experimental – control task contrast

Supplementary Table 4.1 | Group Mean Effects on the Experimental versus Control Block contrast

Cluster number	Region	Cluster size	MNI coordinates (mm)			*z*	*p* (corr.)
			x	y	z		
	Honest						
1	R SFG	22967	14	14	63	7.61	<.001
	R ACC		9	27	25	7.54	
	R paracingulate gyrus		9	27	30	7.42	
	L insula		-36	10	-10	7.32	
	Paracingulate gyrus		0	36	32	7.15	
	L ACC		-7	27	25	7.14	
2	R angular gyrus	1068	61	-51	43	5.57	<.001
	R angular gyrus		56	-49	36	5.40	
	R angular gyrus		58	-54	49	5.27	
	R angular gyrus		58	-45	52	4.81	
	R lateral occipital cortex		65	-59	18	2.92	
	R lateral occipital cortex		47	-62	56	2.82	
3	L lateral occipital cortex	946	-54	-64	41	5.00	.001
	L lateral occipital cortex		-52	-60	39	4.91	
	L angular gyrus		-60	-53	43	4.83	
	L lateral occipital cortex		-49	-62	50	4.81	
	L lateral occipital cortex		-53	-59	47	4.68	
	L angular gyrus		-52	-51	36	4.36	
	Typical lie-tellers						
1	L ACC	23764	-4	27	30	8.41	<.001
	R paracingulate gyrus		2	34	32	8.23	
	R paracingulate gyrus		7	29	34	8.13	
	L paracingulate gyrus		-9	38	28	7.91	
	L paracingulate gyrus		-4	27	36	7.91	
	R paracingulate gyrus		7	29	34	7.71	
2	R angular gyrus	1151	61	-49	49	5.47	<.001
	R angular gyrus		63	-53	36	5.24	
	R angular gyrus		65	-49	38	5.16	
	R angular gyrus		56	-58	49	5.14	
	R angular gyrus		58	-47	36	5.12	
	R lateral occipital cortex		58	-60	45	4.92	
3	L supramarginal gyrus	1058	-63	-49	32	5.48	<.001
	L angular gyrus		-58	-58	43	5.22	
	L angular gyrus		-54	-55	30	4.92	

Supplementary Table 4.1 | *Continued*

Cluster number	Region	Cluster size	MNI coordinates (mm)			*z*	*p* (corr.)
			x	y	z		
	L supramarginal gyrus		-60	-51	45	4.91	
	L angular gyrus		-54	-54	52	4.78	
	L angular gyrus		-65	-53	21	3.78	
	Persistent lie-tellers						
1	R SFG	4402	11	30	63	5.31	<.001
	L SFG		-16	18	68	4.91	
	R SFG		16	22	68	4.90	
	R SFG		9	20	65	4.76	
	L paracingulate gyrus		-7	38	30	4.46	
	L SFG		-7	22	66	4.37	
2	L WM/putamen	1319	-26	12	-10	4.49	<.001
	L WM/putamen/OFC		-25	20	-8	4.00	
	L WM/IFG		-45	11	12	3.93	
	L pallidum		-20	-6	5	3.39	
	L pallidum		-16	-2	5	3.31	
	L central opercular cortex		-43	9	5	3.30	
3	L angular gyrus	752	-49	-58	54	4.05	.004
	L angular gyrus		-56	-57	34	4.01	
	L angular gyrus		-43	-57	16	3.70	
	L lateral occipital cortex		-47	-64	52	3.59	
	L lateral occipital cortex		-54	-60	45	3.56	
	L supramarginal gyrus		-65	-51	20	3.30	
4	R angular gyrus	482	58	-53	35	4.07	.05
	R angular gyrus		58	-49	33	4.03	
	R angular gyrus		62	-47	33	3.78	
	R angular gyrus		49	-50	27	3.48	
	R lateral occipital cortex		58	-58	42	3.45	
	R angular gyrus		63	-49	43	3.24	
	F-test all group means						
1	Paracingulate gyrus	53592	0	36	32	9.27	<.001
	L ACC		-7	27	28	9.07	
	L WM/putamen		-29	10	-10	8.94	
	L paracingulate gyrus		-4	29	37	8.88	
	R ACC		7	29	28	8.77	
	R SFG		14	14	63	8.72	

Supplementary Table 4.1 | *Continued*

Cluster number	Region	Cluster size	MNI coordinates (mm)			*z*	*p* (corr.)
			x	y	z		
2	R angular gyrus	1340	56	-47	56	6.74	<.001
	R angular gyrus		58	-51	49	6.47	
	R angular gyrus		63	-53	42	6.04	
	R angular gyrus		58	-50	54	5.87	
	R angular gyrus		63	-53	36	5.80	
	R angular gyrus		65	-51	27	4.92	
3	L angular gyrus	1278	-56	-57	39	6.01	<.001
	L angular gyrus		-53	-57	30	5.98	
	L angular gyrus		-56	-57	34	5.94	
	L angular gyrus		-61	-55	34	5.82	
	L supramarginal gyrus		-63	-49	32	5.62	
	L angular gyrus		-52	-51	36	5.60	

Note. SFG = superior frontal gyrus; ACC = anterior cingulate cortex; WM = white matter; OFC = orbitofrontal cortex

Chapter 5

Liar, liar, brains on fire:
perceived lie-detectability and children's brain activation patterns during honest and dishonest behavior

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ABSTRACT

The neurobiological correlates of lying may depend on situational factors. We compared three groups of children, one group displaying persistently honest behavior, the second group lied only in a condition with low perceived lie-detectability, and the third group lied persistently in both conditions with perceived low and high lie-detectability. We examined whether the neurobiological correlates of lie-telling and honesty were dependent upon perceived lie-detectability by comparing neural activation in the high lie-detectability condition and the low lie-detectability condition for each group separately. In honest children, high perceived lie-detectability was associated with increased activation in the right lingual gyrus, fusiform gyrus, and cerebellum ($p<.001$). Children who lied only in the low perceived lie-detectability condition showed increased activation in the brainstem in the high lie-detectability condition compared to the low lie-detectability condition ($p=.02$). Persistent lie-tellers showed increased activation in the bilateral anterior cingulate cortex and right paracingulate gyrus in the high lie-detectability condition compared to the low lie-detectability condition ($p<.001$). The significant regions have been implicated in social cognition (honest children), autonomic control (low lie-detectability lie-tellers), and error detection, conflict monitoring or decision-making (persistent lie-tellers). While persistently honest and persistently dishonest children showed similar behavior in the two conditions, high perceived lie-detectability was associated with an increase in brain activation. Situational characteristics thus affect brain activation patterns of both honest and dishonest behavior. Differences in neural activation may underlie persistency and adaptation of behavior across situations, and are informative about subtle differences in individual functioning.

INTRODUCTION

Anyone who claims that he or she always speaks the truth is likely a liar. Although lying is generally considered morally wrong, several studies have shown that people tell one to two lies a day on average (DePaulo, Kashy, Kirkendol, Wyer, & Epstein, 1996; Halevy, Shalvi, & Verschuere, 2014). Dishonesty has been related to a number of situational characteristics. We lie more when we have been ostracized (Poon, Chen, & DeWall, 2013), when we are given little time to think (Shalvi, Eldar, & Bereby-Meyer, 2012), or when we believe our actions are unobserved (Zhong, Bohns, & Gino, 2010). This latter condition (being observed) has large effects on social behavior. The presence of others increases cooperation and generosity (Bateson, Nettle, & Roberts, 2006; Haley & Fessler, 2005), and decreases crime (Ratcliffe, Taniguchi, & Taylor, 2009). Children as young as five years of age share more and steal less when they are being observed by a peer (Engelmann, Herrmann, & Tomasello, 2012), and even the presence of an imaginary but invisible person can prevent children from cheating (Piazza, Bering, & Ingram, 2011). Yet not everyone is equally affected by the presence of an observer. In our study on lie-telling in children, we measured honesty in two conditions: one with low perceived lie-detectability and one with high perceived lie-detectability. We showed that most children lie during the low lie-detectability condition of the task. While the majority of children refrain from lying in the high perceived lie-detectability condition, some children continued to lie despite a high risk of getting detected (Thijssen et al., submitted). For these persistent lie-tellers, the two conditions were not associated with differences in behavior. However, it is unclear whether these children are really unperturbed, and differences are also absent on a neurobiological level. In the present study, we examined whether the neurobiological correlates of lie-telling and honesty are dependent upon perceived lie-detectability in 7- to 10-year old children.

Current knowledge on the neurobiology of deception comes primarily from adult studies that suggest that the prefrontal cortex (e.g. dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC), anterior prefrontal cortex (APFC, or frontal pole)) and anterior cingulate cortex (ACC) play an important role in dishonest behavior (Abe, 2011; Christ, Essen, Watson, Brubaker, & McDermott, 2009; Greene & Paxton, 2009; Ito et al., 2011; Langleben et al., 2002; Lee, Lee, Raine, & Chan, 2010; Spence et al., 2001). These regions have been implicated in cognitive processes related to deception, such as working memory, inhibitory control, and error detection (Abe, 2011). In the few studies in children, deception has been related to activation in the inferior parietal lobe, involved in mentalizing, (Yokota et al., 2013), and the ACC, and frontal pole (Thijssen et al., submitted).

The presence of an observer or audience has not only been related to changes in behavior, but also to changes in neural processing. Finger, Marsh, Kamel, Mitchell, and Blair (2006) asked their participants to read stories in which the participant was the protagonist. In each story, the participant committed a transgression. In some of the stories, the

transgression was committed in front of an audience, but in other stories, the transgression was committed privately. Committing a transgression in front of an audience was associated with increased amygdala activation. Moreover, in a study on the effects of perceived lie-detectability on the neural correlates of dishonesty, participants committed a mock theft and were interrogated about this theft whilst in the MRI scanner (Sip et al., 2013). During some parts of the interrogation, participants believed a lie-detector was activated, while in other parts of the interrogation they were told the lie-detector was turned off. Participants were encouraged to convince the interrogator of their innocence. Lie-telling was associated with increased activity in the right amygdala, inferior frontal gyrus, and the left posterior cingulate cortex. The left temporal pole, the right hippocampus and parahippocampal gyrus were activated during deception when participants believed the lie-detector was turned on (vs. turned off). In the Sip et al. (2013) study, participants were encouraged to lie, which resulted in all participants being dishonest in both conditions (with and without lie-detection). However, in a real life situation it is unlikely that everyone would behave dishonestly during either or both conditions. In the present study, participants were free to choose their tactics, resulting in a more naturalistic measure of dishonest behavior.

Independent of lie-telling, lie-detectability may be emotionally taxing. Therefore, we expected that both persistently honest and persistently dishonest children would show increased activation in limbic areas and the PFC in the high lie-detectability condition compared to the low lie-detectability condition. However, we expect this effect to be stronger for persistently dishonest children, who did not behave according to moral standards than for honest children, who did behave according to moral standards in the low lie-detectability condition. Finally, adapting one's behavior in response to perceived lie-detectability may rely on processes such as theory of mind (ToM) in order to make inferences about the effect of one's behavior on the observer (Amodio & Frith, 2006; Cage, Pellicano, Shah, & Bird, 2013). Therefore, we expected that children who lied in the low lie-detectability condition only would show increased activation in ToM regions during the high lie-detectability condition compared to the low lie-detectability condition.

METHODS

Participants

The present study was embedded within the Generation R Study, a prospective cohort from fetal life onwards in Rotterdam, the Netherlands (Jaddoe et al., 2012; Tiemeier et al., 2012). The honesty task was administered as part of a project on antisocial and prosocial behavior. Participant selection was based on parent reported aggressive behavior (Child Behavior Checklist, administered at age 1.5, 3 and 6 years) (Achenbach & Rescorla, 2000) and prosocial behavior (Strengths and Difficulties Questionnaire, administered at age 6 years) (Goodman,

1997). For children from Dutch origin who had Aggressive Behavior scores available at two or more time points, we distinguished three trajectories of aggression: a high, rising trajectory, an intermediate trajectory, and a low trajectory (Wildeboer et al., 2015). Children in the high, rising trajectory were eligible for participation in the highly aggressive group. Children from the low aggression trajectory who had a minimum score of 14 on the SDQ Prosocial scale (range 5 – 15) were eligible for participation in the prosocial group. Children in the low aggression trajectory with a prosocial score <14, and children in the intermediate trajectory of aggression were eligible for participation in the control group.

Honesty data were available for 174 children of the 291 invited children. Supplementary Figure 5.2 shows a flowchart of participant inclusion. Nine children (5.1%) did not perform the high lie-detectability version of the task because of time constrains and were excluded from analyses. Four children were excluded due to technical problems (2.3%). Forty-nine children (28.2%) were excluded due to excessive motion (>0.5mm mean relative displacement). Finally, as brain activation in response to honesty trials was contrasted against brain activation in response to control trials, we only included children with adequate performance on the control task. Consequently, 10 children (5.7%) were excluded because their score on the control task was not above chance level. As a consequence, fMRI analyses were performed on 99 children (54 girls) of whom 28 children were persistently honest (50% girls), 43 lied only during the low lie-detectability condition (65% girls), and 28 were persistent lie-tellers, who lied during both the low lie-detectability and the high lie-detectability condition (43% girls).

Measures

Honesty task

During the honesty task, which is a child-friendly adaptation based on Greene and Paxton (2009), participants were asked to predict a random computerized event. The participating children were presented with computer images of two dogs. In the experimental condition, participants were requested to predict which dog (left or right) would receive a bone. Participants did not have to report their prediction. Instead, we only asked the children to report their accuracy after each trial. Each correct prediction was rewarded with €0.05. By pushing a button with the middle finger of their dominant hand the children indicated that their response was correct, while a false response was indicated by pushing a button with the index finger. In the control condition, we asked the children to report whether one of the two dogs was wearing a collar. For the control task each correct answer was also rewarded with €0.05. The children received their monetary gain at the end of the scanning session. Figure 5.1 shows details on the timing of the task.

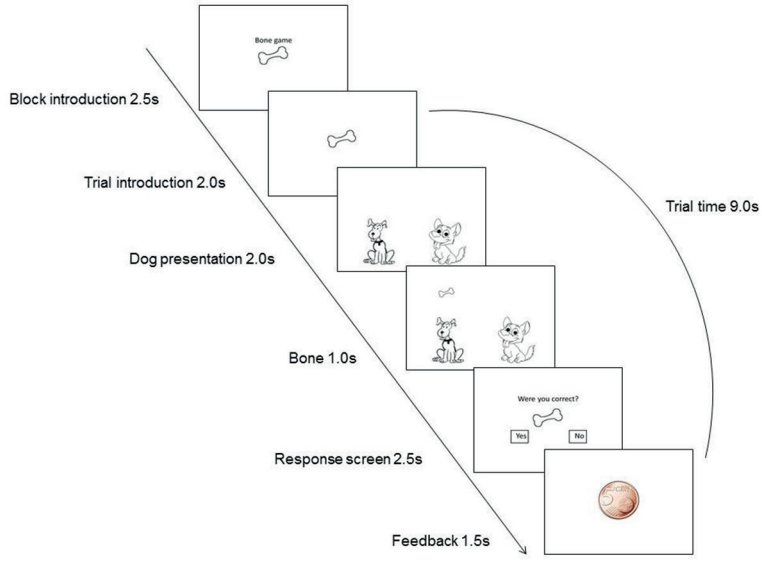

Figure 5.1 | Stimulus presentation

Children were asked to perform the task first in a condition with low perceived lie-detectability and then in a condition with high perceived lie-detectability . The conditions were similar, except that before the start of the condition with high perceived lie-detectability the children were told that the investigators could tell if the children were being honest or not. During the debriefing after the scan, the children were told that this was not true.

The task was coded in PsychoPy version 1.7 (Peirce, 2007), and was administered using a Windows PC. A projector outside of the MRI suite was used to display the task on a large screen located at the front of the MRI system, which was visible through a mirror mounted on the top of the head coil. We used images of six different dogs. For each dog, we created one image with a collar and one image without a collar. The task consisted of 36 trials, presented in 12 blocks of three trials each (6 experimental blocks and 6 control blocks). The allocation of the presentation of the bone was at random. We created four pseudo-random versions of the order of the dogs, two of which started with an experimental block, and two with a control block. Each child was randomly assigned to one of the four versions. All responses were registered using a fiber optic response box (Current Designs, Philadelphia, PA, USA).

Participants who reported improbably high levels of accuracy (one-tailed binomial test, $p<.05$; more than 13 correct guesses (72%) in 18 trials) were classified as dishonest based on chance. All other participants were classified as honest. Based on the two conditions of the task, children were classified as (a) persistently honest (honest in both conditions), (b)

low lie-detectability lie-tellers (dishonest in the low lie-detectability condition only), or (c) persistent lie-tellers (dishonest in both conditions).

Imaging data

A description of the neuroimaging component of the Generation R study has been provided elsewhere (White et al., 2013). Briefly, children were first familiarized with a mock scanning session. MRI scanning was performed on a GE Discovery MR 750 3 T scanner (General Electric, Milwaukee, WI, USA). T1-weighted inversion recovery fast spoiled gradient recalled (IR-FSPGR) sequence was obtained using an 8 channel head coil with the following parameters: TR=10.3 ms, TE=4.2 ms, TI=350 ms, NEX=1, flip angle=16°, readout bandwidth=20.8 kHz, matrix 256x256, imaging acceleration factor 2, and an isotropic resolution of 0.9x0.9x0.9 mm^3. The fMRI-task utilized a gradient-echo blood oxygen level dependent (BOLD) EPI sequence with a TR=2,000 ms, TE=30 ms, flip angle=90°, matrix 64x64 and voxel resolution of 3.6x3.6x4.0 mm^3. The duration of the fMRI paradigm is 6 min. 24 s. (192 TRs).

Covariates

Information on gender, and date of birth was obtained from midwives and hospital registries. Non-verbal intelligence was estimated from the Mosaics and Categories subtest of the Snijders-Oomen Non-Verbal Intelligence Test-Revised (Tellegen, Winkel, Wijnberg-Williams, & Laros, 2005).

Statistical analyses

Group differences in reaction times were examined using ANOVA analyses. Preprocessing and statistical analysis of the imaging data were performed using FSL (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012; Smith et al., 2004). Brain extraction was performed via BET and motion correction was performed using MCFLIRT. Spatial smoothing was applied with a Gaussian kernel of 5 mm (FWHM). Functional MRI data from each child were spatially normalized to their own high resolution T1 image (boundary-based registration (BBR, Greve & Fischl, 2009), 90 degree search) and then to our own child brain template (12 degrees of freedom (DOF), 90 degree search) using FSL's FLIRT registration tool. Given the young age of the sample, it was important to use an age-appropriate template for registration of the functional data to standard space. To construct the age-appropriate template, an iterative, nonlinear approach (Sanchez, Richards, & Almli, 2012) was applied to 130 T1-weighted images (rated as having excellent quality). More information on the construction of the template is provided in Supplementary Text 5.1. For six children, registration to the high-resolution T1 image using BBR failed. For these children, functional imaging data were registered to the high resolution T1 image using an affine transformation, 90 degree search.

After preprocessing, whole-brain statistical analyses were performed at the single-subject level using the general linear model within FSL (FEAT). A design matrix was created and convolved with a gamma hemodynamic response function. Both standard motion parameters and additional motion confound EVs as obtained from fsl_motion_outliers (DVARS, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers) were added to the regression model to address common problems resulting from motion (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). For the GLM, we contrasted experimental vs. control blocks.

Figure 5.2 provides a visual overview of the task and contrasts that were examined. For all groups, a two-sample paired t-test (high lie-detectability – low lie-detectability condition) was performed using FSL's FLAME I + II module to examine the difference between the low lie-detectability condition and the high lie-detectability condition within the different groups. To compare the difference between the low lie-detectability condition and the high lie-detectability condition between the groups, a fixed-effect second level analysis was performed creating a low lie-detectability – high lie-detectability COPE for each participant. This second level analysis was followed by a mixed-effect group analyses using FSL's FLAME I + II module as third level analysis. Age, gender, IQ, and version of the task (i.e., pseudo-randomized order) were used as covariates. Statistical maps were thresholded using clusters determined by $Z>2.3$ and a (corrected) cluster significance threshold of $p<0.05$. Analyses were performed with gray matter mask (Results) and without gray matter mask (Supplementary Table 5.1 and Supplementary Figure 5.3)

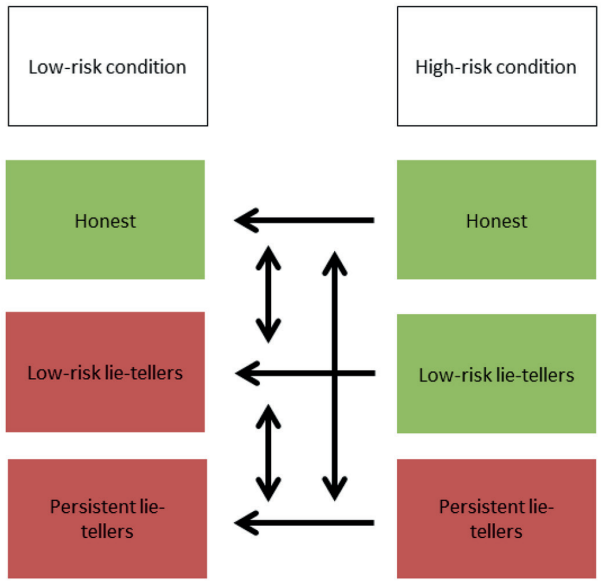

Figure 5.2 | Overview of the conditions and contrasts that were examined

RESULTS

Behavioral data

Information on participant characteristics can be found in Table 5.1. There were no significant differences in reaction times between persistently honest children, low lie-detectability lie-tellers and persistent lie-tellers, $F(2,98)=0.70$, $p=.93$ for low lie-detectability experimental trials, $F(2,98)=0.24$, $p=.79$ for low lie-detectability control trials, $F(2,98)=1.79$, $p=.17$ for high lie-detectability experimental trials, and $F(2,98)=1.03$, $p=.36$ for high lie-detectability control trials.

Table 5.1 | Sample Characteristics

	n	Total group	Honest (*n*=28)	Low lie-detectability lie-tellers (*n*=43)	Persistent lie-tellers (*n*=28)
Girl	99	54 (54.5)	14 (50.0)[a]	28 (65.1)[a]	12 (42.9)[a]
Maternal higher education	99	80 (80.8)	25 (89.3)[a]	33 (76.7)[a]	22 (78.6)[a]
Age	99	8.79 (0.78)	9.15 (0.80)[a]	8.72 (0.78)[ab]	8.63 (0.64)[b]
IQ	90	107.78 (12.99)	110.04 (11.08)[a]	108.65 (12.66)[a]	104.41 (14.83)[a]

Note. In case of categorical variables, numbers represent n(%). Significance was tested using logistic regression analyses. In case of continuous variables, numbers represent M(SD).

All participants responded faster during the high lie-detectability experimental trials ($M=0.86$, $SD=0.19$) compared to the low lie-detectability experimental trials ($M=0.88$, $SD=0.20$), $F(1,96)=4.60$, $p=.03$. There was no difference in reaction time between low lie-detectability and high lie-detectability control trials ($F(1,96)=1.60$, $p=.21$), nor were there any significant group by condition interaction effects on reaction time ($F(2,96)=2.01$, $p=.13$) for experimental trials, and ($F(2,96)=0.67$, $p=.51$) for control trials.

fMRI data

To examine whether the neurobiological correlates of lie-telling and honesty are dependent upon perceived lie-detectability, we performed two-sample paired *t*-tests (high lie-detectability – low lie-detectability condition) for all groups separately. Significant within-subject findings can be found in Table 5.2. In persistently honest children, high perceived lie-detectability was associated with increased activation in the right lingual gyrus, extending the right (temporal) occipital fusiform gyrus and cerebellum compared to low lie-detectability ($p<.001$, See Figure 5.3a). In the low lie-detectability lie-tellers, refraining from lie-telling in the high lie-detectability condition was associated with increased activation in the brain stem compared to lie-telling in the low lie-detectability condition

(*p*=.02, See Figure 5.3b). In persistently dishonest children, high perceived lie-detectability was related to increased activation in the bilateral ACC, extending the right paracingulate gyrus compared to low perceived lie-detectability (*p*<.001, See Figure 5.3c). We did not find any significant between-group differences in high lie-detectability – low lie-detectability activation.

Table 5.2 | Neural Correlates of Lie-Detectability (gray matter mask)

Cluster number	Region	Cluster size	MNI coordinates (mm)			*z*	*p* (corr.)
			x	y	z		
	Honest						
1	Right lingual gyrus	878	34	-46	-2	4.55	< .001
	Right occipital fusiform gyrus		27	-73	-2	3.97	
	Cerebellum		25	-57	-31	3.80	
	Cerebellum		16	-83	-31	3.59	
	Cerebellum		20	-79	-31	3.53	
	Right temporal occipital fusiform gyrus		34	-54	-6	3.50	
	Low lie-detectability lie-tellers						
1	Brain stem	228	4	-31	-8	3.74	.02
	Brain stem		2	-28	-17	3.59	
	Brain stem		9	-30	-15	3.52	
	Brain stem		-2	-26	-10	3.01	
	Brain stem		9	-28	-26	2.98	
	Brain stem		11	-39	-26	2.90	
	Persistent lie-tellers						
1	ACC	913	0	32	23	4.53	< .001
	Right paracingulate gyrus		5	29	36	4.40	
	Right ACC		2	25	19	4.13	
	Left ACC		-2	23	19	4.00	
	Right ACC		9	25	27	3.97	
	Right ACC		2	27	28	3.95	

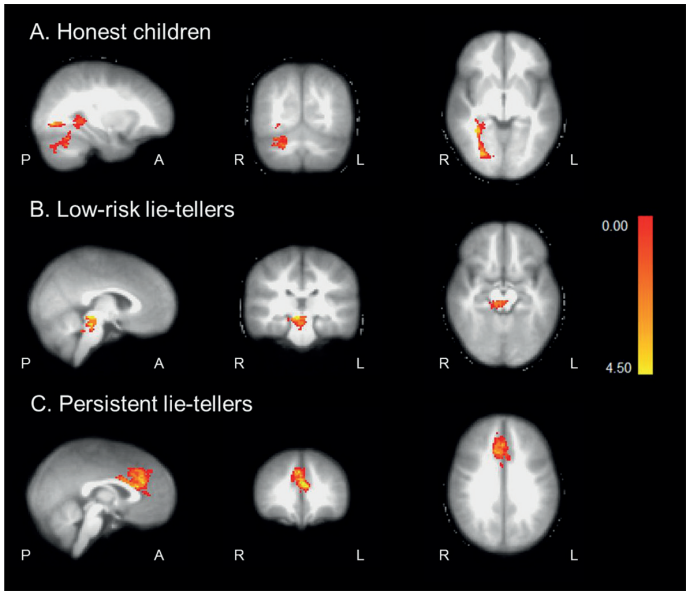

Figure 5.3 | Neural effect of lie-detectability. **A.** In honest children, activity in the right lingual gyrus, fusiform gyrus, and cerebellum was associated with lie-detectability. **B.** Low lie-detectability lie-tellers showed increased activation in the brainstem in the high lie-detectability compared to the low lie-detectability condition. **C.** In persistently dishonest children, lie detectability was associated with increased activity in the bilateral anterior cingulate cortex and right paracingulate gyrus. Effects of lie-detectability did not differ between the groups.

DISCUSSION

The present study examined whether the neurobiological correlates of lie-telling and honesty are dependent upon perceived lie-detectability in 7-to10-year old children. In persistently honest children, high perceived lie-detectability elicited more activation in the right lingual gyrus, extending to the right (temporal) occipital fusiform gyrus and cerebellum. Low lie-detectability lie-tellers showed increased activation in the brainstem when refraining from lying in the high lie-detectability condition compared to lying during the low lie-detectability condition. In children who showed persistent dishonest behavior, high perceived lie-detectability was related to increased BOLD signal in the right bilateral ACC and right paracingulate cortex.

Although persistently honest children behaved according to moral standards in both conditions, their brains were differentially activated when the perceived lie-detectability was high. Compared to low lie-detectability honesty, honesty in the condition with a

high perceived lie-detectability was associated with increased BOLD signal in the right lingual gyrus, extending the right (temporal) occipital fusiform gyrus and cerebellum. The lingual and fusiform gyri are mostly associated with visual processing, but there is evidence that these regions are implicated in social cognition (Geday, Gjedde, Boldsen, & Kupers, 2003; Newsome et al., 2010; Van Overwalle, Baetens, Marien, & Vandekerckhove, 2014). For example, the lingual gyrus has been related to processing threat-related words (Isenberg et al., 1999), and both the fusiform gyrus and cerebellum have been implicated in abstract mentalizing (Schultz et al., 2003; Van Overwalle et al., 2014). This suggests that honest children may infer about the meaning of lie-detectability and its possible social consequences. However, as the lingual gyrus, fusiform gyrus, and cerebellum are mostly involved in processes other than social cognition (such as word and face processing (lingual gyrus and fusiform gyrus), and movement and balance (cerebellum), Cerminara & Apps, 2011; Kanwisher, McDermott, & Chun, 1997; Mechelli, Humphreys, Mayall, Olson, & Price, 2000), this interpretation remains speculative.

In low lie-detectability lie-tellers, refraining from lying in the high perceived lie-detectability condition was associated with increased BOLD signal in the brainstem. The brainstem is involved in autonomic control, and thus may be related to arousal, attention, basic social emotions, and stress (Itoi & Sugimoto, 2010). Feelings of arousal and stress may help explain why the low lie-detectability lie-tellers refrained from lying in the high lie-detectability condition. By design, we compared the neural correlates of honesty to the neural correlates of dishonesty in this group. In general, dishonesty has been related to increased brain activation patterns in comparison to honesty (Abe, 2011; Sip et al., 2013). However, honesty or refraining from lying as a consequence of increased lie-detectability may not be the same as a default honest response. In a study on the neural correlates of honest and dishonest behavior, Greene and Paxton (2009) showed that refraining from lying in dishonest participants is associated with increased BOLD signal in the ACC, PFC, and parietal lobe, regions also involved in dishonest behavior. If similar regions are involved in lying as are in refraining from lying, it is possible that our results do not fully represent the neural correlates of refraining from lying as a consequence of high perceived lie-detectability.

In persistent lie-tellers, we found a stronger BOLD signal in the ACC and paracingulate gyrus in the high lie-detectability condition compared to the low lie-detectability condition. Several studies on deception have implicated the ACC in dishonest behavior (Jiang et al., 2013a; Langleben et al., 2002). The ACC, which receives input from limbic structures and provides output to the prefrontal cortex, has been suggested to play a role in the error detection, conflict monitoring, and the integration of social information, empathy and decision-making (Carter et al., 1998; Lavin et al., 2013). This suggests that when the perceived lie-detectability is high, persistent lie-tellers tellers may engage in more conflict monitoring and error detection when they believe their dishonesty can be detected. Moreover, for

persistent lie-tellers, lie-telling in the high lie-detectability condition may involve a more difficult decision process compared to lying in the low lie-detectability condition. However, we found faster reaction times in the high lie-detectability experimental trials compared to the low lie-detectability experimental trials, which would suggests that the high lie-detectability condition requires a less difficult decision process. However, this difference in reaction time could also be explained by a training effect. However, although the persistent dishonest children did not show behavioral differences in the two conditions, our results suggest that differences in perceived lie-detectability were associated with differences in neural activity in this group.

The increased perceived lie-detectability was related to different regions in the different groups, but we did not find significant differences in high lie-detectability versus low lie-detectability activation between the groups. Thus, the neural effect of increased perceived lie-detectability does not seem to differ significantly between honest children, who behaved according to moral standards, and dishonest children, who did not behave according to moral standards. Moreover, the effect of lie-detectability was similar for persistently honest and dishonest children, who did not change their behavior, and low lie-detectability lie-tellers, who did change their behavior in the high lie-detectability condition. However, as the between-group analysis was a third level subtraction (experimental trials – control trials, high lie-detectability – low lie-detectability, group A – group B), it is possible that the power to find between-group differences in the effect of implied lie-detectability was insufficient.

In their work on situational morality, Van IJzendoorn and Bakermans-Kranenburg suggest that situational characteristics may play a more powerful role in determining moral behavior than genetics, parent-child attachment, temperament or parenting (Van IJzendoorn & Bakermans-Kranenburg, 2014; Van IJzendoorn, Bakermans-Kranenburg, Pannebakker, & Out, 2010). For example, several studies have shown that displaying eyespots to suggest observability increases prosocial behavior (Bateson et al., 2006; Haley & Fessler, 2005). Our results show that demand characteristics of the situation are an important predictor of dishonesty, and that even when manipulations of situational characteristics do not produce behavioral differences, they are still associated with changes in brain activation. The situation, therefore, is a powerful factor in predicting behavioral and/or neural responses, and research may benefit from a stronger consideration of situational effects.

The neuroimaging literature on lying suggests that different types of lies are associated with different neurobiological correlates. For example, Abe et al. (2006) Abe et al. (2006) reported ACC activation in association with false denials, but not with false affirmations. Mameli et al. (2010) used transcranial direct current stimulation to modulate DLPFC function. Stimulation of the DLPFC affected deception about general but not personal knowledge. Moreover, lie-telling may be associated with different patterns of neural activation in different people (Jiang et al., 2013b). Here, we show that demand characteristics of the situation influence brain activation underlying honest and dishonest behavior. Combined,

these findings suggest that brain activation related to dishonest behavior may be highly variable depending on the type of behavior, specific characteristics of the sample, and demand characteristics of the situation.

Some limitations should be noted. We did not ask the children whether they believed their honesty could be evaluated in the high lie-detectability condition. Therefore, it is possible that the children who were dishonest during the high lie-detectability condition continued to lie because they did not believe their honesty could be evaluated. However, children who refrained from lying in the high lie-detectability condition had higher IQ scores compared to the children who continued to lie (Thijssen et al., submitted). Therefore, we expect that this effect is minimal. In order to increase the covert nature of dishonest behavior in the low lie-detectability condition, we did not ask the children to report their prediction. However, as a consequence, we are unable to guarantee that all children defined as honest were actually persistently honest. Furthermore, for the low lie-detectability lie-tellers we found an effect in the brainstem. The brainstem is located close to major arteries and ventricles. Consequently, it is difficult to reliably record fMRI data from this region (Brooks, Faull, Pattinson, & Jenkinson, 2013). Effects found in this region may be due to increased heart rate in the high lie-detectability compared to low lie-detectability condition.

In conclusion, we examined whether the neurobiological correlates of lie-telling and honesty are dependent upon perceived lie-detectability in 7-to 10-year old children. Increased perceived lie-detectability was related to increased BOLD signal in regions implicated in social cognition (right lingual gyrus, (temporal) occipital fusiform gyrus and cerebellum; persistently honest children), autonomic control (brainstem; low lie-detectability lie-tellers), and decision making (bilateral ACC and right paracingulate gyrus; persistent lie-tellers). While their overt behavior was unaffected by the change in situation in persistently honest and persistently dishonest children, the increase in perceived lie-detectability was associated with changes on a neural level. We therefore conclude that situational characteristics (i.e. lie-detectability) affect brain activation patterns of honest and dishonest behavior. Differences in neural activation may underlie persistency and adaptation of behavior across situations, and are informative about subtle differences in individual functioning.

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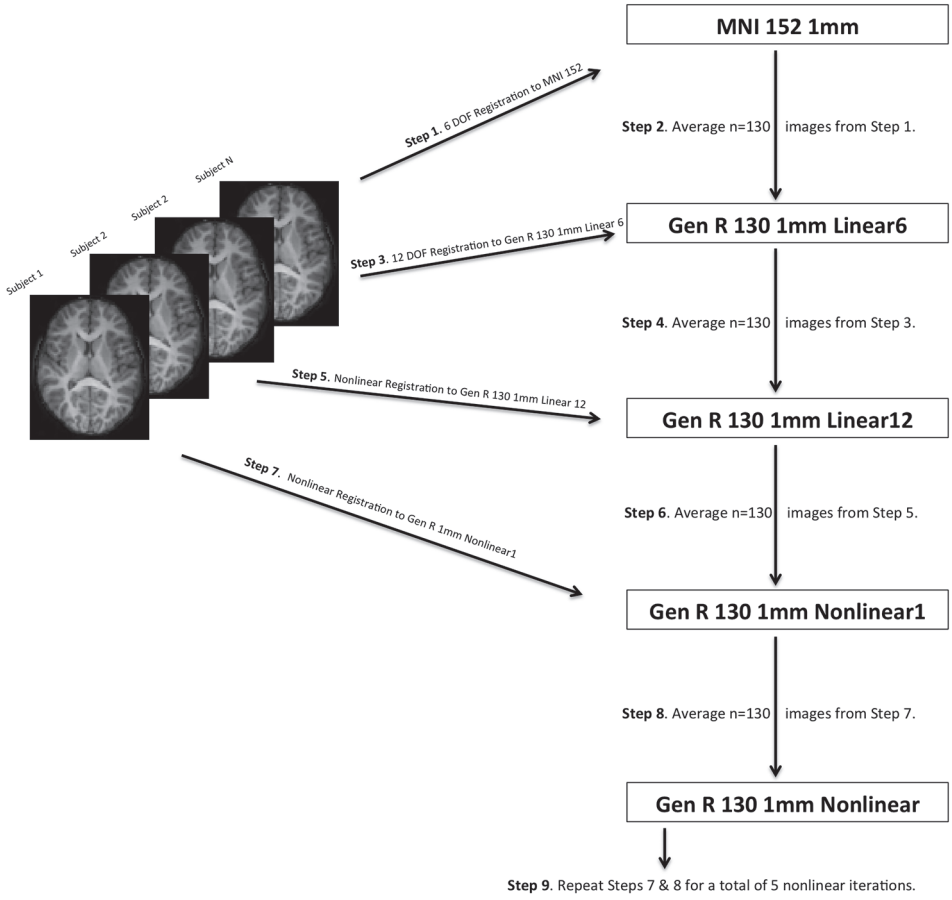
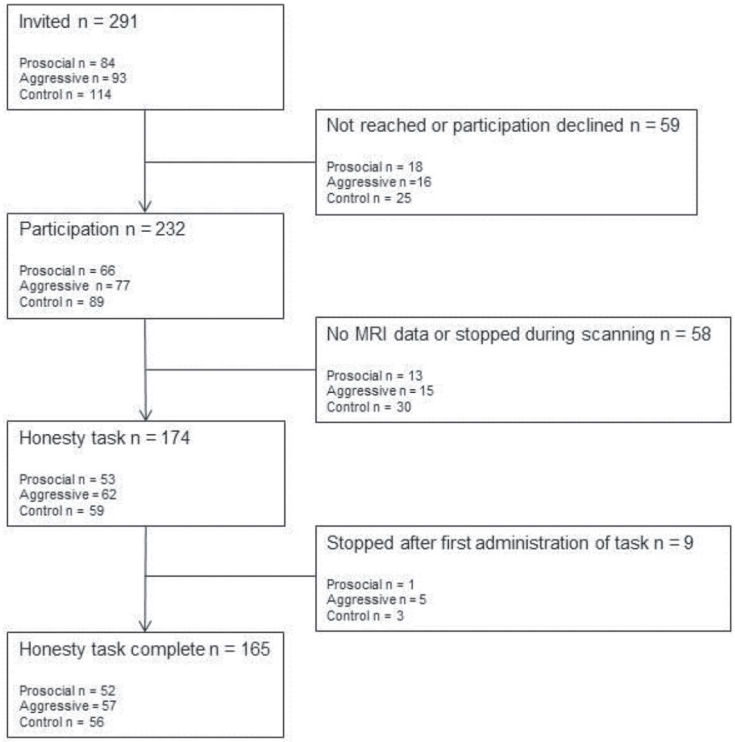
SUPPLEMENTARY TEXT 5.1

Study-specific, age-appropriate template for registration

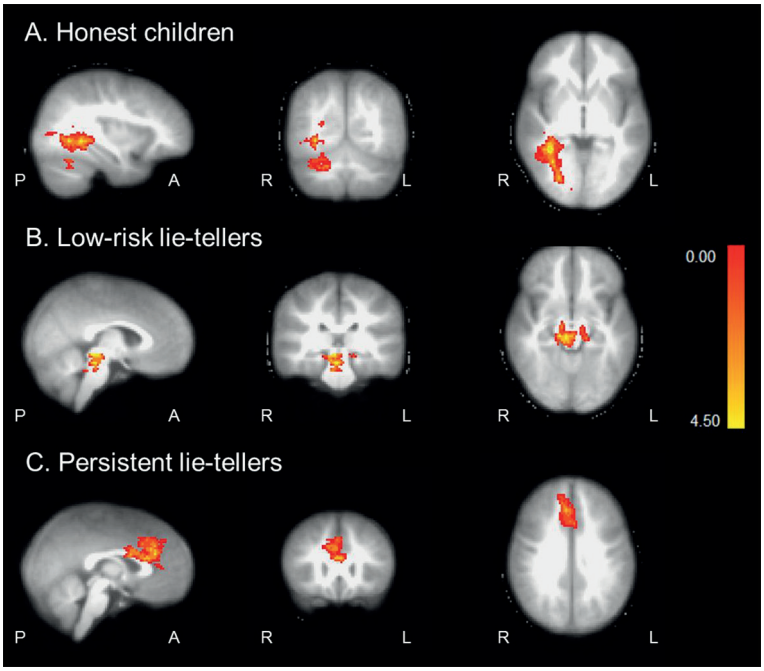
Given the age of the sample, it was important to use an age-appropriate template for registration of the functional data to standard space. One hundred thirty T1-weighted images from children without behavioral problems, also rated as having excellent quality, were used to construct the structural template for registration. An iterative approach using both linear and nonlinear algorithms was used (Sanchez et al., 2012), and is represented graphically in the supplemental data section (Supplementary Figure 5.1). Briefly, T1-weighted images from each of the 130 subjects were first aligned to the MNI-152 1mm brain using a linear, 6 degree of freedom approach (FLIRT). All registered images were then averaged and used as the template brain for the subsequent step, which was a nonlinear registration (FNIRT). Once again, the result from the nonlinear registration was averaged and used as the template for the subsequent iteration. This routine continued for a total of five nonlinear iterations, where it has been shown the template image stabilizes considerably (Sanchez et al., 2012). The result of the fifth and final nonlinear registration was averaged, resampled to 2mm isotropic resolution, and then used as the standard-space template for all FMRI datasets.



Supplementary Figure 5.1 | Study-specific, age-appropriate template for registration



Supplementary Figure 5.2 | Flowchart of participant inclusion



Supplementary Figure 5.3 | Neural effect of lie-detectability (no gray matter mask). **A.** In honest children, activity in the right lingual gyrus, fusiform gyrus, and cerebellum was associated with lie-detectability. **B.** Low lie-detectability lie-tellers showed increased activation in the brainstem in the high lie-detectability compared to the low lie-detectability condition. **C.** In persistently dishonest children, lie detectability was associated with increased activity in the bilateral anterior cingulate cortex and right paracingulate gyrus. Effects of lie-detectability did not differ significantly between the groups.

Supplementary Table 5.1 | Neural Correlates of Lie-Detectability (no gray matter mask)

Cluster number	Region	Cluster size	MNI coordinates (mm)			*z*	*p* (corr.)
			x	y	z		
	Honest						
1	Right WM/temporal occipital fusiform cortex/lingual gyrus	1659	36	-46	-2	4.85	<.001
	Right WM/temporal occipital fusiform cortex/lingual gyrus		34	-58	-2	4.34	
	Right WM/occipital fusiform gyrus		27	-75	-2	4.31	
	Right WM/occipital fusiform gyrus		31	-63	0	4.19	
	Right WM/inferior temporal gyrus		43	-46	-2	3.94	
	Right cerebellum		25	-57	-31	3.8	
	Low lie-detectability lie-tellers						
1	Brainstem	374	4	-31	-8	3.74	<.01
	Brainstem		7	-24	-8	3.68	
	Brainstem		13	-24	-11	3.63	
	Brainstem		2	-28	-17	3.59	
	Brainstem		9	-30	-15	3.52	
	R white matter/thalamus		11	-20	-8	3.39	
	Persistent lie-tellers						
1	ACC	1135	0	32	23	4.53	<.001
	Right paracingulate gyrus		5	29	36	4.40	
	Right ACC		2	25	19	4.13	
	Left ACC		-2	23	19	4.00	
	Right ACC		9	25	27	3.97	
	Right ACC		2	27	28	3.95	

Note. ACC = anterior cingulate cortex.

Chapter 6

Normal variation in early parental sensitivity predicts child structural brain development

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ABSTRACT

Objective: Early caregiving can impact brain structure and function in children. The influence of extreme caregiving experiences has been demonstrated, but studies on the influence of normal variation in parenting quality are scarce. Moreover, no studies to date have included the role of both maternal and paternal sensitivity in child brain maturation. This study examined the prospective relation between mothers' and fathers' sensitive caregiving in early childhood and brain structure later in childhood.

Methods: Participants were enrolled in a population-based prenatal cohort. For 191 families maternal and paternal sensitivity was repeatedly observed when the child was between 1 year and 4 years of age. Head circumference was assessed at 6 weeks, and brain structure was assessed using MRI measurements at 8 years.

Results: Higher levels of parental sensitivity in early childhood were associated with larger total brain volume (adjusted β=.15, *p*=.01) and gray matter volume (adjusted β=.16, *p*=.01) at 8 years, controlling for infant head size. Higher levels of maternal sensitivity in early childhood were associated with a larger gray matter volume (adjusted β=.13, *p*=.04) at 8 years, independent of infant head circumference. Associations with maternal versus paternal sensitivity were not significantly different.

Conclusions: Normal variation in caregiving quality is related to markers of more optimal brain development in children. The results illustrate the important role of both mothers and fathers in child brain development.

INTRODUCTION

Brain development reflects the interplay between genetic and environmental factors (Stiles, 2009). In the last decade, several longitudinal and intervention studies have provided evidence for caregiving influences on child structural and functional brain development (Belsky & de Haan, 2011). These studies have mostly focused on heterogeneous samples with a high-risk for abnormal development due to specific child or parenting characteristics. Studies on the relation between parental care and brain structure in more homogeneous population samples are scarce. Moreover, no studies on the influence of caregiving on child brain structure have used repeated measures of the quality of both maternal and paternal caregiving in early childhood. In the current study, the longitudinal relation of maternal and paternal caregiving with child brain structure is examined in a prospective population-based cohort (*N*=191).

Studies of institutionalized care show that early deprivation is related to reductions in white and gray matter volume, reductions in the volume of the posterior corpus callosum and superior-posterior cerebellum, and to larger amygdala volume compared to children adopted into foster care or healthy controls (Bauer, Hanson, Pierson, Davidson, & Pollak, 2009; Mehta et al., 2009; Sheridan, Fox, Zeanah, McLaughlin, & Nelson, 2012; Tottenham et al., 2010). Moreover, longer exposure to deprivation appears to result in more atypical development (Sheridan et al., 2012; Tottenham et al., 2010). Retrospective studies of exposure to childhood adversities, ranging from chronic family discord to child abuse, have demonstrated reductions in corpus callosum area, gray matter cerebellar and vermis volumes, and hippocampal volume (Riem, Alink, Out, van IJzendoorn, & Bakermans-Kranenburg, in press; Teicher et al., 2004; Walsh et al., 2014). Other studies of high-risk samples defined by preterm birth, socio-economic deprivation, child depression, or maternal substance use show that more sensitive parental care is associated with greater cortical thickness and asymmetry in cortical thickness (Frye, Malmberg, Swank, Smith, & Landry, 2010), and with either smaller (Rao et al., 2010) or larger (Luby et al., 2013; Luby et al., 2012) hippocampal volumes. Moreover, an intervention to enhance maternal sensitivity resulted in greater white matter maturation and connectivity in preterm infants (Milgrom et al., 2010). The results of these high-risk samples may however not be generalizable to the general population due to the relatively extreme caregiving experiences these children were exposed to and due to the large number of potential confounders.

Research on normal variation in parental care and child brain structure in the general population is surprisingly scarce, considering the compelling evidence that early caregiving has a long-term impact on various aspects of child development. Sensitive parental care, characterized by prompt and adequate response to the child's signals and needs (Ainsworth, Bell, & Stayton, 1974), predicts a more secure attachment relationship (Bakermans-Kranenburg, van IJzendoorn, & Juffer, 2003), higher levels of cognitive competence (Kok et

al., 2014; Tamis-LeMonda, Bornstein, & Baumwell, 2001), and less psychological problems (Haltigan, Roisman, & Fraley, 2013; van der Voort et al., 2014). The association between sensitivity and more favorable outcomes in children has been demonstrated for both mothers and fathers (Lewis & Lamb, 2003) One possible mechanism driving the association between parental sensitivity and child development is the impact of sensitivity on brain structure (Belsky & de Haan, 2011) In addition to genetics, environmental influences such as parenting are involved in experience-expectant and experience-dependent processes that can impact for example the pruning and formation of synapses and thus affect brain structural development (Cicchetti, 2002). A recent study has demonstrated this mechanism in adolescents, showing that maternal sensitivity predicted reduced growth in the amygdala and greater thinning of the orbitofrontal cortex four years later (Whittle et al., 2014).

Our study is a unique contribution to the literature in several ways. First, we examined the relation between early parenting and child brain structure in a large and relatively homogeneous sample of healthy children (*N*=191) thus extending previous results to non-disadvantaged families with fewer confounders. Second, we used repeated measures of *observed* parental sensitivity from 1 to 4 years of age to decrease measurement error in the estimated stability of parental sensitivity (Kok, Linting, et al., 2013). Third, we investigated the association of maternal and paternal sensitivity separately and explored whether differences exist in their respective influences on child brain structure. Fourth, we adjusted our analyses for head size at 6 weeks of age and thus accounted for a proxy of brain development immediately after birth and limit the risk of reversed causality. Finally, we examined total brain, white matter, and gray matter volume, and cortical thickness in addition to amygdala and hippocampus volumes to study the relation of parental sensitivity with child brain structure. This approach was chosen as previous studies did not justify testing more specific hypotheses. We expect that parental sensitivity is related to more optimal brain structure in childhood. We do not expect to find differences in the relation between maternal versus paternal sensitivity and child brain structure, because both maternal and paternal sensitivity are related to more favorable child outcomes, and the quality of care may be more influential than whether it is provided by mother of father.

METHODS

The study was embedded within Generation R Study, a prospective cohort investigating growth, development, and health from fetal life onwards in Rotterdam, the Netherlands (Jaddoe et al., 2012). Detailed measurements were obtained in a subgroup of children of Dutch national origin, meaning that the children, their parents, and their grandparents were all born in the Netherlands, to reduce confounding and effect modification (Luijk et al., 2010). The study was approved by the Medical Ethics Committee of the Erasmus Medical Center. Written informed consent was obtained from all adult participants.

From 2009 until 2013, 6- to 10-year-old children from the Generation R Study were invited to participate in a MRI component of the study (White et al., 2013). Approximately 20% of the parents declined to participate. Exclusion criteria for the children were significant motor or sensory disorders, moderate-to-severe head trauma, neurological disorders, claustrophobia, and contraindications to MRI. A total of 246 children participated in the MRI measurement. For 220 children obtained data was of sufficient quality. For 193 children at least one measure of parental sensitivity was available. We excluded 1 twin pair, resulting in 191 parent-child dyads. In the final study sample of 191 parent-child dyads, sensitivity data was available in 188 mother-child and 161 father-child dyads.

A non-response analysis of the 55 parent-child dyads with insufficient data quality or missing data on parenting indicated that they did not differ in gender, parental educational level, hippocampal volume, or amygdala volume. Children excluded from the analyses had less sensitive mothers and smaller total brain, white matter, and gray matter volumes than children included in the analyses (*ps*<.01). Mothers excluded from the analyses were somewhat younger than included mothers (*p*<.05).

Measures

Brain imaging

Infant brain structure. Two indicators of infant brain structure were used in the analyses as baseline measures: ventricular volume and head circumference. Postnatal cranial ultrasounds were performed at 6.6 weeks (*SD*=1.7). To measure the ventricular system, the volume of the ventricular frontal horns, ventricular body, and trigone on both sides was quantified in milliliters. Further details about the ultrasound measurement of the ventricular system have been described elsewhere (Herba et al., 2010; Roza et al., 2008). In addition, the fronto-occipital head circumference of the children was measured. Previous studies have shown that head circumference in infancy is a reasonable proxy for total brain volume (Lindley, Benson, Grimes, Cole, & Herman, 1999).

Child brain structure. Magnetic resonance imaging was performed when the children were approximately 8 years (*M*=8.04, *SD*=0.93). Children were familiarized with the MRI environment during a mock session. Images were acquired on a 3Tesla scanner (750 Discovery, GE Healthcare, Milwaukee, WI) using an eight-channel head coil. Following a three-planer localizing scan, a high-resolution T1 inversion recovery fast-spoiled gradient recalled sequence was acquired in the sagittal plane with the following parameters; TE=4.24ms, T1=350ms, TR=10.26ms, NEX=1, flip angel=16˚, and resolution 0.9mm3 isotropic.

Image processing. Cortical reconstruction and volumetric segmentation was performed with the Freesurfer image analysis suite 5.1. The technical details of these procedures are described in prior publications (Reuter, Schmansky, Rosas, & Fischl, 2012). Briefly, processing

included intensity normalization, removal of non-brain tissue, automated Talairach transformation into standard space, segmentation of the cortical and subcortical white and gray matter structures, tessellation of the gray/white matter boundary, automated topology correction, and surface deformation. Once the cortical models were complete, the images underwent surface inflation, registration to a spherical atlas, and the parcellation of the cerebral cortex into units based on gyral and sulcal structure. Cortical thickness was calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface (Fischl & Dale, 2000). At the scan site and after processing through FreeSurfer, structural images and segmentation quality were rated. Images were excluded if initial T1 scans were rated as unusable or poor, if images could not be processed by FreeSurfer, or if images had poor segmentation quality (*n*=26). We excluded scans with unusable hippocampus or amygdala segmentation (*n*=20) from the hippocampal and amygdala analyses, respectively. The following volume measurements were analyzed: total brain, gray matter, white matter, hippocampus (adjusted for total brain volume), and amygdala (adjusted for total brain volume). Volume measures were z-standardized to facilitate interpretation.

Sensitivity

Parental sensitivity was observed when the children were 1, 3, and 4 years of age. At 1 year of age, child and primary caregiver (86% mothers) were observed in a 5-minute free play session and a 5-minute psychophysiological assessment (data not presented in this paper) using Ainsworth's 9-point rating scales for *Sensitivity* and *Cooperation*.(Ainsworth et al., 1974) An overall sensitivity score was created by aggregating the standardized subscale scores of both subscales in both tasks (Kok, Linting, et al., 2013). The intercoder reliability (intraclass correlation coefficient [ICC], single measure, absolute agreement) was .65 (Luijk et al., 2011).

At 3 and 4 years of age, parents and children were observed during four 3- to 4-minute tasks that were too difficult for the child: building a tower and an etch-a-sketch task. At 3 years of age the child participated with the primary caregiver (82% mothers). At 4 years the child participated with both parents (response rate: 91% mothers, and 100% fathers). Sessions were coded using the revised Erickson 7-point rating scales for *Supportive presence* and *Intrusiveness* (Egeland, Erickson, Clemenhagen-Moon, Hiester, & Korfmacher, 1990). Overall sensitivity scores for the 3- and 4-year measurements were created by aggregating the standardized subscale scores of both subscales in both tasks (reversing the Intrusiveness scores). The ICC was .81 for the 3 year measurement and .84 for the 4 year measurement (Kok, van IJzendoorn, et al., 2013). Sensitivity scores of the primary caregivers were only moderately stable across 1 to 4 years of age (range between .10 and .32). The highest stability estimates were observed across short (i.e., 1 to 2 year) intervals. Concordance between fathers and mothers at 4 years was .12. Three composite sensitivity scores for *maternal and paternal* sensitivity combined, and for *maternal* sensitivity and *paternal* sensitivity separately were created by averaging the relevant standardized sensitivity scores.

Covariates

Analyses were controlled for child gender and age at MRI measurement to adjust for gender and age differences in brain maturation (De Bellis et al., 2001). Further, covariates were included in the regression model when they generated a change in the effect estimate of the predictor of ≥5%. Handedness at age 8 did not meet the criterion and was therefore not included as a covariate. All analyses were additionally controlled for parental educational level, child emotional and behavior problems, and child IQ. Parental educational level was reported at the intake of the Generation R Study. We used the Child Behavior Checklist (CBCL/1½ – 5) to measure child behavioral and emotional problems (Achenbach & Rescorla, 2000). Mothers and fathers completed the CBCL when the children were on average 3 years old (*M*=36.4 months, *SD*=0.73). Child nonverbal IQ was assessed at age 6 with two subtests of the Snijders-Oomen non-verbal intelligence test (SON-R 2.5-7): Mosaics and Categories (Tellegen, Winkel, Wijnberg-Williams, & Laros, 2005).

Statistical Analyses

First, we tested whether maternal and paternal sensitivity were associated with child brain measurements at age 8 with separate linear regression analyses, adjusted for gender, age at MRI measurement, parental educational level, behavioral and emotional problems, and IQ. In a second step, the regression analyses were additionally adjusted for infant brain structure (head circumference or ventricular volume). The corresponding analyses were also performed for the combined measure of maternal and paternal sensitivity. We tested the difference in associations of maternal and paternal sensitivity with child brain measurements using the Fisher r-to-z transformation of partial correlations.

In addition, we performed vertex-wise whole-brain exploratory analyses of the association between parental sensitivity and cortical thickness, using FreeSurfer's QDEC. Regions for which cortical thickness was significantly associated with parental sensitivity were determined using fully adjusted general linear models with all relevant covariates. To correct for the effect of multiple comparisons, a cluster based Monte Carlo simulation was performed using 10,000 iterations and $p \leq .05$. A surface-based Region Of Interest (ROI) was manually created for brain areas that were significantly related to parental sensitivity and utilized to extract the mean cortical thickness within the specific ROI for every participant. ROIs were then residualized for total brain volume. Linear regression was used to examine whether parental sensitivity explained variance in the extracted ROIs independent of covariates.

Missing data were imputed with the predictive mean matching method in IBM SPSS Statistics, version 21.0.0.1 for Windows. Due to missing data on child behavioral and emotional problems (4%), child IQ (6%), head circumference (6%), and ventricular volume (14%), we generated five imputed data sets for the regression analyses including covariates

(*N*=191). Analyses conducted with the pooled, imputed data yielded similar results as the analyses with the complete data.

RESULTS

The sample consisted of 50% girls. The average birth weight was 3563 g (*SD*=508). Children had an average score of 20.3 (*SD*=14.5) on behavioral and emotional problems, and an average IQ of 106.3 (*SD*=14.0). The mean age of mother and father at intake was 31.8 (*SD*=3.7) and 33.8 (*SD*=4.6) respectively; 60.7% of parents had a high educational level.

Significant gender differences were found in brain volume at age 8: girls had a smaller total brain volume, $t(189)=4.29$, $p<.001$, white matter volume, $t(189)=4.93$, $p<.001$, and gray matter volume, $t(189)=3.54$, $p<.001$, than boys. Children with highly educated parents had larger total brain volume, $t(189)=-2.31$, $p=.02$, and gray matter volume, $t(189)=-2.52$, $p=.01$, than children from families with a low/medium educational level. Total sensitivity was higher in families with a high parental educational level compared to families with a low/medium educational level, $t(189)=-1.98$, $p=.05$. Mothers of children with more behavioral and emotional problems were less sensitive, $r=-.20$, $p=.01$.

In Supplementary Table 6.1 associations between parental sensitivity and infant brain structure at 6 weeks and brain volume at 8 years are presented. Infant head circumference was not associated with parental sensitivity. A larger head circumference at 6 weeks was associated with larger total brain, white, and gray matter volume at 8 years ($r=.56$, $p<.001$; $r=.55$, $p<.001$; $r=.51$, $p<.001$, respectively), and larger hippocampal and amygdala volumes ($r=.21$, $p<.01$; $r=.18$, $p<.05$, respectively). Larger ventricular volume at 6 weeks was associated with larger total brain, white, and gray matter volume ($r=.25$, $p<.01$; $r=.27$, $p<.01$; $r=.20$, $p<.01$, respectively) at 8 years.

Maternal sensitivity

More maternal sensitivity significantly predicted larger gray matter volume (adjusted model: B=.23, 95% CI .02-.44, β=.13, $p=.03$) at 8 years, over and above covariates including infant head circumference (see Table 6.1). Maternal sensitivity predicted total brain volume at trend level (adjusted model: B=.19, 95% CI -.01-.39, $p=.06$). No significant associations were found between maternal sensitivity and white matter, amygdala, or hippocampal volumes.

Paternal sensitivity

Effect estimates for the association between paternal sensitivity and children's brain volume at 8 years were similar to the estimates for the association with maternal sensitivity but not significant (see Table 6.1).

Table 6.1 | Associations Between Parental Sensitivity and Child Brain Volume at 8 Years

	Total brain volume (*z*-score)			White matter (*z*-score)			Gray matter (*z*-score)			Hippocampusa (*z*-score)			Amygdalaa (*z*-score)		
	B (95% CI)	β	*p*	B (95% CI)	β	*p*	B (95% CI)	β	*p*	B (95% CI)	β	*p*	B (95% CI)	β	*p*
Maternal sensitivity[b] (*z*-score) *427 assessments (188 mothers)*															
Model I[c]	.24(.01-.46)	.14	≤.05	.16(-.06-.38)	.09	.15	.27(.04-.50)	.16	≤.05	.00(-.22-.22)	.00	.98	.14(-.11-.39)	.09	.28
Model II[c]	.19(-.01-.39)	.11	.06	.12(-.08-.31)	.07	.25	.23(.02-.44)	.13	≤.05	.00(-.21-.22)	.00	.97	.14(-.10-.39)	.09	.26
Paternal sensitivity[b] (*z*-score) *208 assessments (161 fathers)*															
Model I[d]	.15(-.08-.38)	.09	.21	.14(-.08-.37)	.09	.21	.14(-.10-.38)	.08	.24	-.17(-.38-.04)	-.14	.11	.06(-.19-.32)	.04	.62
Model II[d]	.14(-.06-.34)	.08	.18	.13(-.06-.33)	.08	.19	.13(-.08-.34)	.08	.23	-.17(-.38-.03)	-.14	.10	.06(-.19-.31)	.04	.64
Maternal and paternal sensitivity[b] (*z*-score) *635 assessments (191 parent:188 mothers and 161 fathers)*															
Model I[e]	.33(.06-.59)	.16	≤.05	.25 (-.01-.51)	.12	.06	.35(.08-.63)	.17	≤.05	-.13(-.39-.13)	-.08	.31	.15(-.15-.45)	.08	.33
Model II[e]	.31(.07-.55)	.15	≤.05	.23 (.00-.47)	.11	.05	.34(.09-.59)	.16	≤.01	-.14(-.40-.11)	-.09	.27	.14(-.16-.43)	.07	.37

Note. In Model I all values were adjusted for child gender and age at MRI-measure, parental educational level, child behavioral and emotional problems at 3 years, and child IQ at 6 years. In Model II values were additionally adjusted for infant head circumference. Full case analyses yielded similar results.

[a] Adjusted for total brain volume

[b] Composite sensitivity scores were created by taking the mean of standardized sensitivity scores at 1, 3, and 4 years.

[c] *n*=168

[d] *n*=145

[e] *n*=171

Maternal and paternal sensitivity

Comparison of partial and semi-partial correlations for the separate linear regression models for maternal sensitivity and paternal sensitivity controlled for covariates did not yield significant results. The interaction between maternal and paternal sensitivity was not significant either (data not shown). Due to the similarity of associations for maternal and paternal sensitivity, the sequence of analyses was repeated for the more robust combined measure of maternal and paternal sensitivity.

A higher level of parental sensitivity predicted larger total brain volume (adjusted model: B=.31, 95% CI .07-.55, β=.15, *p*=.01) and gray matter volume (adjusted model: B=.34, 95% CI .09-.59, β=.16, *p*=.01) at 8 years, over and above covariates including infant head circumference (see Table 6.2). Parental sensitivity predicted larger white matter volume at trend level (adjusted model: B=.23, 95% CI .00-.47, β=.11, *p*=.05). The interaction between gender and sensitivity on child brain volume was not significant (data not shown). The association between total sensitivity and brain volume was not accounted for by sensitivity at one specific timepoint (see Supplementary Table 6.2) and additional adjustment for ventricular volume yielded similar results (data not shown). Sensitivity analyses showed that results remained essentially unchanged if the model was adjusted for paternal report of child behavior problems, and also if the model was adjusted for parental income instead of education (data not shown).

Sensitivity was associated with thicker cortices in a cluster including the left precentral, postcentral, and caudal middle frontal gyrus (1473 mm^2, max vertex X=-52.5, Y=-6.6, Z=38.5; adjusted model: B=.13, 95% CI .07-.19, β=.28, *p*<.001). In the right hemisphere, sensitivity was related to thicker cortices in a partly overlapping cluster, including the precentral, caudal middle frontal, and rostral middle frontal gyrus (2511 mm^2, max vertex X=39.1, Y=-9.6, Z=60.8; adjusted model: B=.11, 95% CI .05-.17, β=.26, *p*<.001). Results are presented in Table 6.2 and Figure 6.1. Results were similar when the model was additionally adjusted for child handedness (data not shown).

Table 6.2 | Associations Between Parental Sensitivity and Cortical Thickness at 8 Years

	Right hemisphere			Left hemisphere		
	Precentral gyrus[a] (mm)			Precentral gyrus[a] (mm)		
	B (95% CI)	**β**	***p***	**B (95% CI)**	**β**	***p***
Maternal and paternal sensitivity (z-score) *635 assessments (191 parents, of which 188 mothers and 161 fathers)*						
Model I	.11(.05-.17)	.26	<.001	.13(.07-.19)	.29	<.001
Model II	.11(.05-.17)	.26	<.001	.13(.07-.19)	.28	<.001

N*ote:* In Model I all values were adjusted for child gender and age at MRI-measure, parental educational level, child behavioral and emotional problems at 3 years, and child IQ at 6 years. In Model II values were additionally adjusted for infant head circumference. Full case analyses yielded similar results.

[a] Adjusted for total brain volume

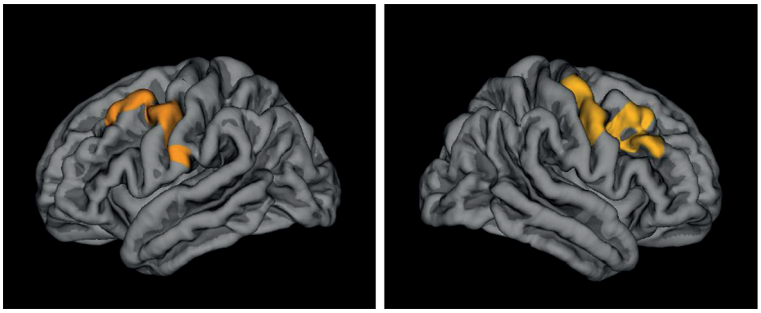

Figure 6.1 | The association between parental sensitivity in early childhood and cortical thickness at age 8 years. The colored areas on the surface map represent clusters of thicker cortices related to higher levels of parental sensitivity. In the left hemisphere the cluster covered the precentral, postcentral, and caudal middle frontal gyrus (adjusted β=.28, $p<.001$). In the right hemisphere the cluster covered the precentral, caudal middle frontal, and rostral middle frontal gyrus (adjusted β=.26, $p<.001$).

DISCUSSION

In this population-based study the prospective relation between parental sensitivity and child brain structure in a low-risk sample was examined. We found that parental sensitivity in early childhood was positively associated with markers of more optimal brain development at age 8, including a larger total brain volume, larger gray matter volume, and thicker cortices in the precentral, postcentral, caudal middle frontal, and rostral middle frontal gyrus. The associations were similar for maternal and paternal sensitivity, and independent of infant head circumference. Our results extend the evidence for an association between the quality of early caregiving and child brain development provided by studies in high-risk populations.

Our results indicate that parental sensitivity is related to more global measures of brain volume, but not to the volume of specific subcortical structures. This is in line with a review concluding that most solid evidence exists for the association between early caregiving and global brain volume (Belsky & de Haan, 2011). Although some studies have found specific associations between early caregiving experiences and hippocampal volume (Luby et al., 2013; Luby et al., 2012; Rao et al., 2010; Riem et al., in press) or amygdala volume (Mehta et al., 2009; Tottenham et al., 2010; Whittle et al., 2014), our results suggest a more general effect of early caregiving on brain volume. Considering the fact that parental sensitivity is related to a variety of child developmental outcomes, including cognitive, behavioral, and social domains (Bakermans-Kranenburg et al., 2003; Haltigan et al., 2013; Kok et al., 2014;

Mintz, Hamre, & Hatfield, 2011; Tamis-LeMonda et al., 2001; van der Voort et al., 2014), a global effect of sensitivity on the brain seems plausible.

The associations were similar for mothers' and fathers' parenting sensitivity. Although the similarity in effect sizes could reflect a spill-over of the quality of maternal sensitivity on paternal sensitivity (Erel & Burman, 1995), our results suggest that fathers' sensitivity is no less important for child brain development than mothers' sensitivity. Our results appear to imply that the quality of caregiving is most important for brain development and not dependent on the person providing this care. The role of the father in caregiving and in child development is less well-studied than the role of the mother, and the influence of fathers appears underestimated. Our findings emphasize the importance of the whole family system for optimal child development.

Parental sensitivity was a significant predictor of child brain volume independent of infant head size, suggesting the association cannot be explained by underlying biological vulnerability. Due to the design with only one measure of child brain volume we cannot preclude reverse causality, but our findings are in concordance with recent experimental studies showing that parental sensitivity can result in differences in brain development in both infants and adolescents (Milgrom et al., 2010; Whittle et al., 2014).

Several mechanisms may explain the association between parental sensitivity and child brain volume. First, early sensitive caregiving may reduce exposure to and experience of stress in children. Earlier studies have demonstrated the role of early caregiving in stress-regulation (Kertes et al., 2009; Lupien, McEwen, Gunnar, & Heim, 2009). Early life stress can potentially disrupt the organization and development of the brain by loss of neurons, influence on dendritic branching or spine density (Lupien et al., 2009), or a decrease in neurogenesis , which can be an adaptive short-term response with maladaptive long-term consequences (Baker et al., 2013). Secondly, the stimulation provided by sensitive caregivers implies a supportive environment that facilitates increases in brain volume and cortical thickness. Previous studies have demonstrated that practice in cognitive or motor tasks results in changes in brain structure, including gray matter (Draganski et al., 2004) and cortical thickness (Haier, Karama, Leyba, & Jung, 2009).

Despite our study's strengths, including the use of repeated measure of parental sensitivity, the inclusion of both mothers and fathers, and the generalizability of results to typically developing children, the results must be interpreted within the context of some limitations. First, we did not have repeated measures of brain volume and could therefore not study brain development in relation to parental sensitivity. A baseline measure of brain volume in early infancy using MRI is possible (Rifkin-Graboi et al., 2015) but our university ethics committee does not approve MRI studies in typically developing children under age six. Second, although we controlled for a number of potential confounders, we cannot preclude residual confounding. Third, the behavioral implication of the relation between parental sensitivity and brain volume, and how brain volume might mediate the influence

of earlier parenting on later child development, are empirical questions. The precise understanding of the relation between brain volume and functioning in early childhood is still emerging (Hart & Rubia, 2012).

Our study demonstrates that normal variation in parental sensitivity in early childhood is related to brain volume at age 8. These findings are in line with robust evidence that caregiving is essential for child development in cognitive, behavioral, and social domains. The similarity in results for maternal sensitivity and paternal sensitivity emphasizes the importance of including both maternal and paternal caregiving in research on the relation between early parental care and child brain development.

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Supplementary Table 6.1 | Associations Between Infant Brain Structures (6 wks), Parental Sensitivity (1 – 4 yrs), and Child Brain Volume (8 yrs)

	Ventricular volume (6w)	Maternal and Paternal Sensitivity	Maternal Sensitivity	Paternal Sensitivity	Total brain volume (8y)	White matter (8y)	Gray matter (8y)	Hippocampus[a,b] (8y)	Amygdala[a,b] (8y)
Head circumference (6w)	.30**	.05	.08	.02	.56**	.55**	.51**	.21**	.18*
Ventricular volume (6w)		.03	-.02	.13	.25**	.27**	.20*	-.04	.07
Maternal and Paternal Sensitivity			.88**	.60**	.20**	.16*	.22**	.06	.18*
Maternal Sensitivity				.14	.18*	.13	.20**	.12	.20*
Paternal Sensitivity					.11	.10	.10	-.04	.08
Total brain volume (8y)						.94**	.97**	.60**	.47**
White matter (8y)								.56**	.44**
Gray matter (8y)								.58**	.46**
Hippocampus (8y)									.51**

*p<.05;**p<.01.
[a] Unadjusted for total brain volume
[b] n=171

Supplementary Table 6.2 | Associations Between Parental Sensitivity (1 – 4 yrs) and Child Brain Volume (8 yrs)

	Total brain volume	White matter	Gray matter	Amygdala[a]	Hippocampusa
Sensitivity					
1y primary caregiver[b] (*n*=154)	.11	.07	.13	.11	-.01
3y primary caregiver[c] (*n*=184)	.11	.12	.10	.10	.05
4y mother (*n*=143)	.15	.11	.17*	.07	.01
4y father (*n*=157)	.11	.10	.11	.10	.02

* $p<.05$
[a] Adjusted for total brain volume
[b] Including 135 mothers, 19 fathers
[c] Including 152 mothers, 32 fathers

Chapter 7

General discussion

In this thesis, we studied the neurobiological correlates of externalizing behavior as well as prosocial behavior in school-age children. This chapter provides a general discussion of the research findings, speculative considerations, and practical implications along with suggestions for future research.

MAIN FINDINGS

Brain morphology of aggressive behavior and prosocial behavior

In chapter 2 we examined the brain morphology of aggressive behavior in 566 6-to 9-year old children using a multi-informant approach. We performed vertex-based whole brain analyses to examine the association between aggressive behavior and cortical thickness, cortical surface area and cortical gyrification. Additionally, we examined the association between aggressive behavior and amygdala and hippocampal volume. We showed that aggression is related to decreased amygdala volume in typically developing children. Moreover, childhood aggressive behavior is associated with decreased sensorimotor cortical thickness and widespread decreased right hemisphere gyrification. Finally, aggressive behavior was associated with cortical thickness in regions that are part of the default mode network (superior and middle frontal gyrus, precuneus, posterior cingulate cortex), with positive associations in girls and negative associations in boys.

In chapter 3, we examined the association between cortical thickness and prosocial behavior in 464 6- to 9-year old children. Prosocial behavior was related to a thicker cortex in a cluster that includes part of the left superior frontal and rostral middle frontal cortex. Gender moderated the association between prosocial behavior and cortical thickness in a cluster including the right rostral middle frontal and superior frontal cortex as well as in a cluster including the right superior parietal cortex, cuneus and precuneus, with positive associations in boys, and negative associations in girls. Our results suggest that prosocial behavior is associated with increased cortical thickness in regions related to theory of mind (superior frontal cortex, rostral middle frontal cortex cuneus, and precuneus) (Carrington & Bailey, 2009; Singer, 2006) and inhibitory control (superior frontal and rostral middle frontal cortex) (Durston et al., 2002). These findings are in accordance with developmental studies showing associations between theory of mind or executive function and prosocial behavior (Eggum et al., 2011; Eisenberg, Fabes, & Spinrad, 2007).

Interestingly, in chapter 2 and 3 we describe gender-by-cortical thickness interaction effects on the association between cortical thickness and aggressive behavior or prosocial behavior in the frontal and medial posterior cortex. Analyses for aggressive behavior were corrected for prosocial behavior and vice versa. In girls, the association between cortical thickness and prosocial behavior was negative, while the association between cortical thickness and aggressive behavior was positive. For boys, the findings were opposite:

increased prosocial behavior was associated with a thicker cortex, and increased aggressive behavior was associated with cortical thinning. The frontal and medial posterior cortex have been implicated in theory of mind (Carrington & Bailey, 2009; Singer, 2006), and executive functioning (Durston et al., 2002), and are part of the default mode network, a network involved in self-reflection and moral reasoning (Whitfield-Gabrieli & Ford, 2012). Our results suggest that cortical thickness in these regions may be involved in social behavior more generally, and differently in boys and girls. These gender differences may represent a difference in maturation: the social brain matures faster in girls than in boys (Mutlu et al., 2013), and children with elevated psychiatric traits (e.g. conduct problems) are suggested to have a delayed or aberrant cortical maturation (De Brito et al., 2009; Dennis & Thompson, 2013). In childhood, cortical thickness increases until it reaches its peak thickness around puberty (Giedd et al., 1999). Thereafter, processes such as synaptic pruning result in cortical thinning. If the cortex of the girls in our sample already shows thinning, the positive association between cortical thickness and aggression, and the negative association between cortical thickness and prosocial behavior, may be explained by delayed maturation in high aggressive/low prosocial girls. Due to their inferred slower maturation compared to girls, the boys in our sample may still show cortical increase. If the boys with high levels of aggressive behavior/low levels of prosocial behavior showed a delayed cortical maturation, this would explain why they have a thinner cortex compared to the low aggressive/high prosocial boys. However, our studies described cross-sectional data and cannot provide information on neurodevelopmental trajectories underlying aggressive/prosocial behavior. Longitudinal studies are necessary to examine the neurobiological development of aggressive and prosocial behavior.

Correlates of lie-telling

In contrast to a trait-like interpretation of behavior as deployed in chapter 2 and 3, externalizing and prosocial behavior may also be the product of the demand-characteristics of the situation. Situational characteristics may be specifically important for dishonest behavior. For example, while honesty is considered morally superior to lying, in some situations (such as when receiving an undesirable gift) telling a lie may be the appropriate response ("thank you, it is beautiful!"). But even in the case of antisocial lies, telling a lie or two a day may be the norm (DePaulo, Kashy, Kirkendol, Wyer, & Epstein, 1996; Halevy, Shalvi, & Verschuere, 2014). Problems arise when lying becomes habitual and is persistent across situations (Talwar & Crossman, 2011).

In chapter 4, we aimed to differentiate between honest children, children who showed typical lie-telling behavior and children who lied more persistently, and examined what demographic, cognitive, social and neurobiological factors are associated with lie-telling behavior. To this aim, we examined lying in a condition with low perceived lie-detectability and a condition with high perceived lie-detectability. In both conditions, behaving

dishonestly increased monetary gain. However, in the condition with high perceived lie-detectability, the children were made to believe that the experimenter could see whether they were being dishonest. Children who were honest in both conditions were referred to as honest or persistently honest. Children who were dishonest in one condition only -the largest group- were referred to as typical lie-tellers (chapter 4) or low lie-detectability lie-tellers (chapter 5). Participants who lied in both the low lie-detectability and the high lie-detectability condition were considered persistent lie-tellers. Figure 7.1 gives a visual presentation of the conditions, groups and contrasts examined in chapter 4 and 5.Of the 163 children, 75% lied in the low lie-detectability condition, compared to 34% in high-risk condition. Results of a discriminant analysis showed that persistent lie-tellers could be discriminated from other children based on gender (more boys), lower age, lower IQ, less effortful control, and lower educated mothers. Compared to honest children and persistent lie-tellers, typical lie-tellers were more likely to be girls and to come from families with higher income. The neurobiological data mimicked our behavioral results in showing similar neural patterns in honest children and typical lie-tellers, while persistent lie-tellers differed from typical lie-tellers and honest children in showing less brain activation of the anterior cingulate cortex and frontal pole during the task. These findings suggest that persistent lie-tellers engage in less conflict monitoring and error detection during lie-telling.

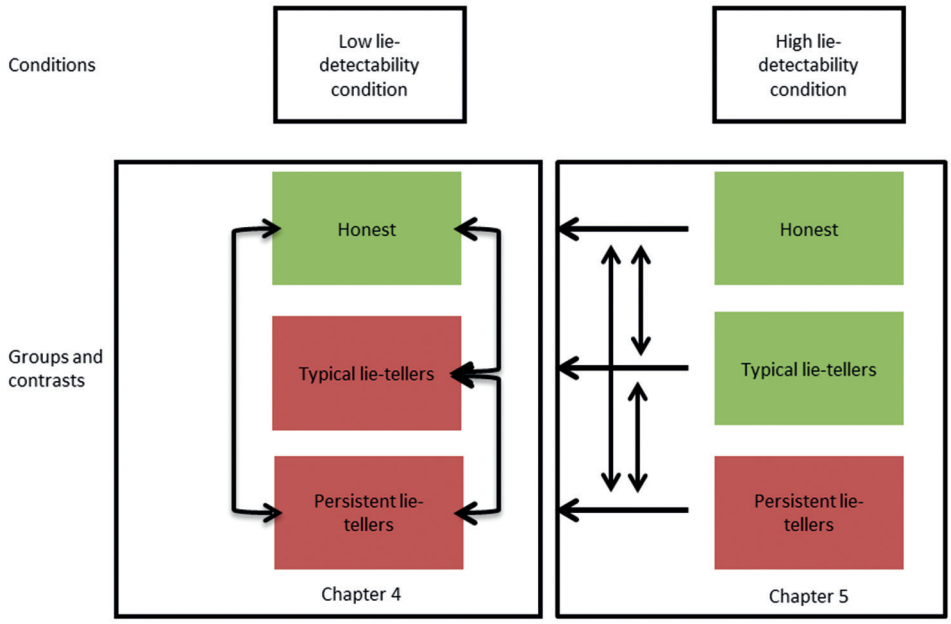

Figure 7.1 | Contrasts examined in chapter 4 and chapter 5

In chapter 5 we examined whether the neurobiological correlates of lie-telling and honesty are dependent upon perceived lie-detectability by comparing neural activation in the high lie-detectability condition with the low lie-detectability condition for each group separately. Compared to low perceived lie-detectability, high perceived lie-detectability was related to increased activation in regions implicated in social cognition (persistently honest children), autonomic control (low lie-detectability lie-tellers), and error detection, conflict monitoring or decision making (persistent lie-tellers). While persistently honest as well as persistently dishonest children showed similar behavior in the low lie-detectability condition and high lie-detectability condition, for both groups of children the high perceived lie-detectability condition was associated with an increase in brain activation compared to the low perceived lie-detectability condition. Thus, situational characteristics (i.e. lie-detectability) may affect brain activation patterns of honest and dishonest behavior.

As many children lied during the low lie-detectability condition, our results suggest that some lying may be normative. Persistent lie-telling, however, was associated with a more adverse demographic and cognitive background. Interestingly, we did not find any association between lie-telling and prosocial, aggressive or moral behavior. This suggests that lie-telling, and even lie-telling that is persistent across situations, may be the product of cognition or temperament rather than moral or social behavior. Alternatively, there are many different types of lies (simple lies, complicated lies, lies for rewards, lies to avoid punishment etc.), and it is possible that other types of lie-telling are associated with social and moral characteristics. As lie-telling in the present study was not harmful to other people, the absence of an association between lie-telling and social behavior and moral development may not be unexpected. Future studies examining lie-telling in the context of externalizing or antisocial behavior may want to adopt a paradigm that assesses social lying (lying to a person instead of a computer) or harmful lying.

Interestingly, in chapter 4 we showed that persistent lie-tellers showed decreased activity in the ACC during the low lie-detectability condition compared to honest children and typical lie-tellers. In chapter 5, we show that for persistent lie-tellers, dishonesty in the high lie-detectability condition is associated with increased activity in the ACC compared to dishonesty in low lie-detectability condition. These results suggest that persistent lie-tellers may require a higher level of risk in order to engage the ACC in the same degree as typical lie-tellers or honest children. To test this, we compared low lie-detectability brain activation patterns of honest children and typical lie-tellers with high lie-detectability brain activation patterns of persistent lie-tellers. These results did not show any significant differences between persistent lie-tellers and honest children or typical lie-tellers, and thus provide support for this explanation.

In his paper "A trio of concerns", Jerome Kagan (2007) argues that the current focus on biological explanations for psychological phenomena may be misplaced, as factors such as socioeconomic background may explain a much larger amount of variance. To

test this claim within the present study, we extracted the averaged residualized BOLD signal for all voxels that showed a larger BOLD signal for the honest children compared to the persistent lie-tellers during the low lie-detectability condition. We also extracted the averaged residualized BOLD signal for all voxels that showed a larger BOLD signal for the typical lie-tellers compared to the persistent lie-tellers. Over and above age, effortful control, IQ, gender, and maternal education, which together explained 32.5% of variance, the averaged residualized fMRI data explained 14.0% of additional variance between the honest children and persistent lie-tellers. Over and above age, effortful control, IQ, gender, and family income, which together explained 18.8% of variance, the averaged residualized fMRI data explained 13.5% of additional variance between typical lie-tellers and persistent lie-tellers. These findings counteract Kagan's claim and suggests that fMRI data may provide important information on processes involved in deception, but also provide additional evidence for predicting dishonesty in children.

In chapter 4 we show that, even in the low-risk condition, persistent lie-tellers differ from typical lie-tellers in brain activation patterns of dishonest behavior, suggesting neural differences even when the behavior (i.e. lie-telling) is the same. Moreover, in chapter 5 we show that the neural correlates of lie-telling also depended on demand characteristics of the situation. Thus, brain activation related to behavior may be highly variable depending on the specific type of behavior, characteristics of the sample and demand characteristics of the situation.

Early caregiving and brain morphology

Studies in high-risk samples show that early caregiving can impact brain structure and functioning in children (Sheridan, Fox, Zeanah, McLaughlin, & Nelson, 2012; Tottenham et al., 2010). In chapter 6, we examined the prospective relation between quality of sensitive caregiving of mothers and fathers in early childhood and child brain structure later in childhood in a population-based sample. Higher levels of maternal sensitivity in early childhood were associated with a larger gray matter volume at 8 years of the child's age, independent of infant head circumference. No significant differences were found between associations of maternal versus paternal sensitivity with child brain volume. Higher levels of combined parental sensitivity in early childhood were associated with a larger total brain volume and a larger gray matter volume at 8 years of child's age, independent of infant head size. Parental sensitivity was associated with a thicker cortex in a cluster including the left precentral, postcentral and caudal middle frontal cortex as well as in a cluster including the right precentral and middle frontal gyrus.

Intervention studies have shown that increasing parental sensitivity decreases externalizing behavior in children (Bakermans-Kranenburg, Van IJzendoorn, Pijlman, Mesman, & Juffer, 2008; Moss et al., 2011; Van Zeijl et al., 2006). While we find positive associations between parental sensitivity and global markers of brain morphometry,

parental sensitivity was also associated with increased cortical thickness in the bilateral frontal and sensorimotor cortex. Interestingly, in chapter 2 we show that aggressive behavior is associated with decreased cortical thickness in similar regions. In order to examine whether bilateral frontal and sensorimotor cortical thickness may mediate associations between parental sensitivity and aggressive behavior, we first examined whether parental sensitivity and its associated clusters were associated with aggressive behavior in the present sample. While parental sensitivity was associated with aggressive behavior, we did not find associations between cortical thickness and aggressive behavior, and thus did not find proof for a mediation effect.

METHODOLOGICAL CONSIDERATIONS

Multiple comparisons in whole-brain analyses

By computing the positive predictive value (PPV, the probability that a positive research finding is a true positive), Ioannidis (2005) showed that many published research findings are actually false positives. However, when positive results are replicated by another study, the PPV is enhanced (Moonesinghe, Khoury, & Janssens, 2007). Nevertheless, neuroimaging findings are often reported without replication. This may be especially problematic when neuroimaging studies adopt a whole-brain approach rather than a hypothesis-based ROI analysis. While whole-brain analyses provide better specificity than ROI analyses, they do require a large number of comparisons, thus increasing the risk of false positives. To decrease this risk, whole-brain analyses should always be corrected for multiple comparisons (Hagler, Saygin, & Sereno, 2006). However, correction for multiple comparisons may not completely rule out the risk of chance findings.

Throughout this thesis we have performed whole-brain analyses to assess associations between brain structure/function and behavior. Using a Monte-Carlo simulation with 10.000 iterations and $p<.05$ we corrected for multiple comparisons. Moreover, in an attempt to deal with concerns about false positive findings, we complemented our structural analyses by bootstrapping the whole-brain analysis using 500 samples of 200 participants (Efron & Tibshirani, 1993). For each vertex, *p-values* were averaged to generate an overall result. While this method does not involve replication by a different study sample, it does provide some evidence regarding the sample specificity of the positive results, as results would need to be replicated in several of the subsamples in order for the averaged *p-value* to reach statistical significance.

Multiple informants

In psychological and pedagogical research, validity of behavioral measures remains an important topic. We can distinguish between different types of behavioral measurements,

such as observational measurements and self (or other) reports. Collecting observational data is time consuming, and as data is often collected in the lab, results may be situationally dependent and hard to generalize to real-life situations. While questionnaires or interviews often refer to real-life situations, self-reports may be biased as participants may provide socially desirable answers (Choi & Pak, 2005). In the case of young children or individuals with intellectual disabilities, self-reports may be problematic as participants may not possess the cognitive abilities to reflect on their own behavior (Edelbrock, Costello, Dulcan, Kalas, & Conover, 1985). In this case, parent or teacher reports may provide useful sources of information. However, parental or teacher psychological state may affect the way they experience and thus report on the participant's behavior (Durbin & Wilson, 2012). Moreover, parents and teachers may report more stereotypic gender roles (Mills & Rubin, 1990). Observational data is believed to be less biased than self or other reported data (Kagan, 2007). However, in a sample as large as the Generation R cohort, it may not always be feasible to collect all data of interest by means of observation.

In order to increase the validity of our measure of childhood aggression, in chapter 2 we implemented a multi-informant approach by combining parent-reported and child-reported data on child behavior. Generally, agreement between parent and child informants tends not to exceed 0.20 (Kraemer et al., 2003). Low levels of agreement between informants suggest that childhood functioning is best conceptualized as the separate and combined influences of children's actual characteristics, the context in which children are observed, the perspectives (or biases) of the informants, and error of measurement. Kraemer at al. (2003) therefore suggest that the choice of informants should be based on consideration of the contexts and perspectives that influence the characteristic under investigation. Weaknesses of one informant should be compensated by strengths of another. While parents observe their child only in the home environment, the child itself can report on his or her behavior in all contexts. Furthermore, children provide information on how they perceive themselves, while parents provide an other-report.

We performed whole-brain vertex-wise analyses using the multiple-informant aggressive behavior measure, which we then repeated for the parent and child data separately. After correction for multiple comparisons, the multiple-informant measure of childhood aggressive behavior showed significant associations with cortical thickness in regions that did not appear associated with the parent or child data separately. Interestingly, the regions that were associated with the multiple-informant score, but not with the parent and child data separately, were associated with the separate scores before correction for multiple comparisons. These findings suggest that the separate informant scores may be clouded by noise, and that combining data from multiple-reporters may provide a measure that is more precise than its subparts. In behavioral neuroscience, many studies still use one informant to report on behavior. Future studies on the association between behavior and brain morphology may want to follow our approach.

Confounding

In epidemiology, a confounding variable is an extraneous variable that distorts the association between the dependent variable and the independent variable. For example, intelligence may play an important role in explaining associations between brain structure/function and prosocial or externalizing behavior. When analyses are not adjusted for the effects of confounders, associations between independent and dependent variables may be under- but typically overestimated. However, partly due to relatively small sample sizes, many neuroimaging studies do not take into account important confounding variables.

In the studies described in chapter 2 and 3 in this thesis, we have attempted to deal with confounding by correcting for possible confounding variables in two consecutive steps. During the whole-brain analyses, the association between behavior and cortical thickness was adjusted for gender, age and IQ. Significant clusters were then extracted and imported to SPSS, where we examined the effects of other possible confounding variables using linear regression analyses. We choose to employ this strategy, as it is not possible to assess the effects of multiple categorical variables in Freesurfer's QDEC, the program we used to perform vertex-based whole-brain analyses. Moreover, by using linear regression analyses we were able to quickly assess what variables were related to both our independent and dependent variables, a prerequisite for a variable to be a confounding variable, and to assess the percentage of change the confounding variable inflicted on the effect estimate of the independent variable. However, if confounding variables play a large role in explaining the association between independent and dependent variable, our strategy may have insufficiently adjusted the association between independent and dependent variable. After performing the whole-brain analysis, results are corrected for multiple comparisons by using a Monte-Carlo simulation with 10.000 iterations and $p<.05$. This Monte Carlo correction takes into account the size and *p-value* of significant clusters. It is possible that some of the significant regions would not have survived correction for multiple comparisons if the confounders were added to the whole-brain analysis, while the association between these regions and aggressive or prosocial behavior remain significant after adjustment for confounders in the linear regression analyses.

CLINICAL AND SCIENTIFIC IMPLICATIONS

When examining the association between behavior and brain functioning or structure in adults, associations may be caused by the behavior itself (reversed causality) and/or environmental influences (e.g. lead exposure, substance abuse). Studying the neurobiological correlates of behavior in children increases the chance of identifying structures that are involved in the etiology of that behavior (Sterzer & Stadler, 2009). Finding structures involved in etiology of behavior is an important goal of research, as information on causal factors are important

for treatment and intervention. In our study on the neurobiological correlates of aggressive behavior, we replicated the association between aggressive behavior and amygdala volume found in adults (Ermer, Cope, Nyalakanti, Calhoun, & Kiehl, 2012; Pardini, Raine, Erickson, & Loeber, 2013; Siever, 2008). However, some other well reported associations between brain morphology and aggression or antisocial behavior, such as in the ACC and orbitofrontal cortex (OFC) (Yang & Raine, 2009), were not replicated. A lower amygdala volume, but perhaps not cortical thinning in the ACC and OFC, may therefore play an important role in the development of antisocial behavior. The amygdala has been implicated in emotional processes, such as emotional learning, and may facilitate attention to salient cues (Phelps & LeDoux, 2005). However, before treatment and intervention programs may profit from this finding, future studies may want to longitudinally examine whether amygdala volume is associated with the development of aggressive and antisocial behavior.

While there may be individual differences in externalizing and prosocial behavior, behavior may also be affected by situational factors. For example, children donate more money to a charity when they are subtly prompted by the experimenter (Van IJzendoorn, Bakermans-Kranenburg, Pannebakker, & Out, 2010). Both in experimental and field settings, displaying eyespots to suggest observability increases prosocial behavior (Bateson et al., 2006; Haley & Fessler, 2005). In chapter 4 we show that lie-telling is also highly dependent on demand characteristics of the situation. While many children lie when they believe they will not get caught, most children refrain from lying when they think there is a high risk of lie-detectability. These findings may be used to create situations that evoke moral behavior in the school, home or on the streets. Even the suggestion of observability may deter children from committing a transgression.

In chapter 6 we show that quality of parenting in the first years of life is associated with brain morphology later in life. Low quality parenting has often been associated with adverse outcomes, and these association may thus be explained by changes in brain morphology. While we did not find evidence for an explanatory effect of cortical thickness in the association between parental sensitivity and aggressive behavior, changes in brain morphology may still partly explain parenting effects found on child externalizing behavior. Future studies may want to examine the mechanisms through which parenting may affect brain morphology. For example, in early childhood, parents play an important role in their children's stress-regulation (Lupien, McEwen, Gunnar, & Heim, 2009). Increased stress hormone cortisol in early childhood has been associated with aberrant brain structure and functioning later in life (Callaghan, Sullivan, Howell, & Tottenham, 2014).

CONCLUDING REMARKS

This thesis describes our work on the neurobiological correlates of externalizing and prosocial behavior in six-to ten-year old children. We showed that aggressive behavior and prosocial behavior are associated with cortical thickness in similar regions, differently in boys and girls. While we were able to replicate the association between aggressive behavior and amygdala volume found in adults, some other well reported associations between brain morphology and aggression or antisocial behavior, such as findings in ACC and OFC, were not replicated. It is possible that these associations become apparent later in development, or are a consequence rather than a cause of the aggressive behavior and co-occurring environmental influences. While our work has provided important insights on the neuroanatomical correlates of externalizing and prosocial behavior in children, the brain morphometric studies described in this thesis are of cross-sectional design. Longitudinal studies are necessary to model development.

Our results on lie-telling show that behavior and its neural correlates are highly dependent upon demand characteristics of the situation. While many children may behave inappropriately when they believe no one is watching, they may change their behavior when they think they are being observed. These findings may be used to create situations that evoke less aggressive or immoral behavior in the school, home or on the streets. However, not all children are equally affected by situational changes. We showed that the persistently dishonest children are characterized by a more adverse demographic and cognitive background. Increasing parenting skills may be an important area of intervention, as increased parental sensitivity is associated with markers of more optimal brain development.

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Appendices

Summary

This thesis describes a series of studies on the neurobiological correlates of externalizing and prosocial behavior in six-to ten- year old children. Chapter 1 provides an outline and describes the background and aims of our work. The studies described in this thesis are embedded in the Generation R study, a prospective cohort from fetal life onwards in Rotterdam, the Netherlands. We describe both structural (chapter 2, 3, and 6) and functional neuroimaging studies (chapter 4 and 5) on the association between externalizing and prosocial behavior and the brain, and examine behavior both from a trait-like perspective (chapter 2 and 3) as well as from a state-like perspective (chapter 4 and 5).

In chapter 2 and 3, we explored the neuroanatomical correlates of aggressive and prosocial behavior in six-to-nine year old children. Aggressive behavior was associated with smaller amygdala, but not smaller hippocampal volume. Moreover, aggression was associated with a thinner cortex in sensorimotor regions, and widespread decreased right hemisphere gyrification. Finally, aggressive behavior was associated with cortical thickness in regions that are part of the default mode network (superior and middle frontal gyrus, precuneus, posterior cingulate cortex), with positive associations in girls and negative associations in boys. Prosocial behavior was related to a thicker cortex in a cluster that included part of the left superior frontal and rostral middle frontal cortex. Moreover, the association between prosocial behavior and cortical thickness was different for boys and girls in similar regions as reported in relation to aggressive behavior (superior and middle frontal cortex, cuneus, precuneus).

In chapter 4, we aimed to differentiate between honest children, children who showed typical lie-telling behavior and children who lied more persistently, and we examined what demographic, cognitive, social and neurobiological factors are associated with lie-telling behavior. To this aim, we observed lying in a condition with low perceived lie-detectability and a condition with high perceived lie-detectability. Children who were honest in both conditions were referred to as honest or persistently honest. Children who were dishonest in one condition only -the largest group- were referred to as typical lie-tellers (chapter 4) or low lie-detectability lie-tellers (chapter 5). Participants who lied in both the low lie-detectability and the high lie-detectability condition were considered persistent lie-tellers. Of the 163 children, 75% lied in the low lie-detectability condition, compared to 34% in high-risk condition. Persistent lie-tellers could be discriminated from other children based on gender (more boys), lower age, lower IQ, less effortful control, and lower educated mothers. Compared to honest children and persistent lie-tellers, typical lie-tellers were more likely to be girls and to come from families with higher incomes. The neurobiological data mimicked our behavioral results in showing similar neural patterns in honest children and typical lie-tellers, while persistent lie-tellers differed from typical lie-tellers and honest

children in showing less brain activation of the anterior cingulate cortex and frontal pole during the task. These findings suggest that persistent lie-tellers engage in less conflict monitoring and error detection during lie-telling.

In chapter 5 we examined whether the neurobiological correlates of lie-telling and honesty are dependent upon perceived lie-detectability by comparing neural activation in the high lie-detectability condition with the low lie-detectability condition for each group separately. Compared to low perceived lie-detectability, high perceived lie-detectability was related to increased activation in regions implicated in social cognition (persistently honest children), autonomic control (low lie-detectability lie-tellers), and decision making, error detection or conflict monitoring (persistent lie-tellers). While persistently honest as well as persistently dishonest children showed similar behavior in the low lie-detectability condition and high lie-detectability condition, in both groups of children the high perceived lie-detectability condition was associated with an increase in brain activation compared to the low perceived lie-detectability condition. Thus, situational characteristics (i.e. lie-detectability) affect brain activation patterns of honest and dishonest behavior.

In chapter 6, we describe the prospective relation between mothers' and fathers' sensitive caregiving in early childhood and brain morphology later in childhood. As quality of parenting has been associated with child externalizing behavior, examining the association between parental sensitivity and brain morphology may provide information on mechanisms through which parenting influences child behavior. More parental sensitivity in early childhood was associated with larger total brain volume and gray matter volume at 8 years, controlling for infant head size. Moreover, sensitivity was associated with a thicker cortex of the bilateral precentral, and middle frontal gyri. The results illustrate the important role of parents in child brain development.

In chapter 7, the main findings of the studies reported in this thesis are summarized. Moreover, the chapter describes methodological considerations, and practical implications along with suggestions for future research.

Samenvatting

Dit proefschrift beschrijft een reeks van studies over de neurobiologische correlaten van externaliserend en prosociaal gedrag in kinderen van zes tot tien jaar oud. In hoofdstuk 1 wordt een overzicht gegeven van de achtergrond en de doelen van ons werk. De studies die in dit proefschrift beschreven worden, zijn onderdeel van het Generation R onderzoek, een prospectief cohortonderzoek vanaf het vroege foetale leven tot in de jongvolwassenheid in Rotterdam. We beschrijven zowel structurele (hoofdstuk 2, 3 en 6) als functionele neuroimaging studies over de associatie tussen externaliserend gedrag en prosociaal gedrag en het brein, en onderzoeken gedrag zowel vanuit een trait-perspectief (persoonlijkheidkenmerk) als vanuit een state-perspectief (afhankelijk van de situatie).

In hoofdstuk 2 en 3 hebben we de neuro-anatomische correlaten van agressief en prosociaal gedrag onderzocht in kinderen van zes tot negen jaar. Agressief gedrag was gerelateerd aan een kleiner volume van de amygdala, maar niet aan een kleiner volume van de hippocampus. Agressie was gerelateerd aan een dunnere cortex in sensomotorische gebieden, en aan verminderde corticale vouw in grote delen van de rechter hemisfeer. Bovendien was agressie gerelateerd aan corticale dikte in gebieden die onderdeel uitmaken van het default mode netwerk (superieure en middelste frontale gyrus, precuneus en posterieure cingulate gyrus). Associaties tussen deze gebieden en agressie waren positief in meisjes, maar negatief in jongens. Prosociaal gedrag was gerelateerd aan een dikkere cortex in een cluster dat de linker superieure frontale en rostrale middelste frontale gyrus omvat. Bovendien vonden we verschillende associaties tussen prosocial gedrag en corticale dikte voor jongens en meisjes in gebieden waar we ook geslachtsverschillen vonden in relatie tot agressief gedrag (superieure en middelste frontale gyrus, cuneus en precuneus).

In hoofdstuk 4 hebben we een poging gedaan om onderscheid te maken tussen eerlijke kinderen, kinderen die normatief lieggedrag laten zien en kinderen die meer persistent liegen. We hebben onderzocht welke demografische, cognitieve, sociale en neurobiologische factoren geassocieerd zijn met verschillen in lieggedrag. Om dit voor elkaar te krijgen, hebben we liegen geobserveerd in een conditie met een lage mate van waargenomen leugendetectie en in een conditie met een hoge mate van waargenomen leugendetectie. Kinderen die in beide condities eerlijk waren, werden beschouwd als eerlijke kinderen, ofwel persistent eerlijke kinderen. Kinderen die slechts in één conditie oneerlijk waren -de grootste groep kinderen-, werden beschouwd als normatief oneerlijke kinderen. Kinderen die tijdens beide condities oneerlijk gedrag vertoonden, werden beschouwd als persistent oneerlijke kinderen. Van de 163 kinderen was 75% oneerlijk tijdens de conditie met een lage mate van waargenomen leugendetectie. In de conditie met een hoge mate van waargenomen leugendetectie was 34% oneerlijk. Kinderen die persistent oneerlijk waren, konden worden onderscheden van de andere kinderen op basis van hun geslacht (meer jongens), en op basis van hun jongere leeftijd, lagere IQ, lagere regulatief vermogen

en lagere opgeleide moeders. In vergelijking met eerlijke kinderen en persistent oneerlijke kinderen waren normatief oneerlijke kinderen vaker meisjes en kwamen ze uit families met een hoger inkomen. De neurobiologische data komen sterk overeen met de gedragsdata. Deze data laten namelijk zien dat eerlijke kinderen en normatief oneerlijke kinderen gelijke activatie patronen vertonen tijdens de conditie met een laag risico op leugendetectie. In vergelijking met de eerlijke kinderen en normatief oneerlijke kinderen, laten de persistent oneerlijke kinderen minder activatie zien in de anterieure cingulate gyrus en de rechter frontale pool. Deze bevindingen suggereren dat persistent oneerlijke kinderen een lagere mate conflict monitoring en error detectie laten zien gedurende oneerlijke gedrag.

In hoofdstuk 5 hebben we onderzocht of de neurobiologische correlaten van eerlijk en oneerlijk gedrag afhankelijk zijn van de mate van waargenomen leugendetectie. We hebben dit gedaan door de neurale activiteit van de conditie met een hoge mate van waargenomen leugendetectie te vergelijken met de activiteit tijdens de conditie met een lage mate van waargenomen leugendetectie voor de drie groepen apart. In vergelijking met de conditie met een lage mate van waargenomen leugendetectie, was de conditie met een hoge mate van leugendetectie gerelateerd aan een verhoogde activatie in gebieden die gerelateerd zijn aan sociale cognitie (eerlijke kinderen), autonomische controle (normatief oneerlijke kinderen) en besluitvorming, error detectie of conflict monitoring (persistent oneerlijke kinderen). Terwijl eerlijke kinderen en persistent oneerlijke kinderen hetzelfde gedrag vertonen in de conditie met een lage alsmede een hoge mate van waargenomen leugendetectie, laten beide groepen verhoogde activatie zien tijdens de conditie met een hoge mate van waargenomen leugendetectie in vergelijking met de conditie met een lage mate van waargenomen leugendetectie. Karakteristieken van de situatie (bijvoorbeeld mate van waargenomen leugendetectie) hebben dus invloed op de hersenactivatie patronen van eerlijk en oneerlijk gedrag.

In hoofdstuk 6 beschrijven we de prospectieve relatie tussen de sensitieve opvoeding van moeders en vaders tijdens de vroege kindertijd en de structuur van de hersenen van hun kinderen later in de kindertijd. Omdat kwaliteit van de opvoeding is gerelateerd aan externaliserend gedrag van het kind, kan onderzoek naar de associatie tussen sensitieve opvoeding en morfologie van de hersenen informatie geven over de mechanismen waarlangs opvoeding het gedrag van het kind beïnvloedt. Meer sensitiviteit van de ouders tijdens de vroege kindertijd was geassocieerd met een groter volume van de hersenen en een groter volume van de grijze stof, zelfs wanneer gecontroleerd wordt voor de omtrek van het hoofd tijdens de vroege kindertijd. Sensitiviteit was bovendien gerelateerd aan een dikkere cortex in de bilaterale precentrale en middelste frontale gyri. De resultaten laten de belangrijke rol zien die ouders spelen bij de ontwikkeling van de hersenen van hun kinderen.

In hoofdstuk 7 worden de belangrijkste bevindingen van het proefschrift samengevat. Bovendien beschrijft het hoofdstuk methodologische overwegingen, praktische implicaties en suggesties voor vervolgonderzoek.

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About the Author

Sandra Thijssen was born on July 12, 1989 in Rotterdam, the Netherlands. She grew up in Krimpen aan den IJssel where she attended the Krimpenerwaard College. She graduated from high school in 2007, after which she studied Psychology at the Erasmus University of Rotterdam. In 2009, she spent a semester at the Swinburne University of Melbourne. In 2011 she obtained her Master's Degree in Brain and Cognition (cum laude). In the summer of 2011 she started as a PhD at the Department of Pedagogical Sciences of Erasmus University of Rotterdam and the Generation R Study group at the Erasmus Medical Center, under supervision of prof. dr. Marinus H. van IJzendoorn, prof. dr. Henning Tiemeier, prof. dr. Marian J. Bakermans-Kranenburg and dr. Tonya White. The results of her PhD project are presented in this thesis.

PhD portfolio

Name PhD student:	Sandra Thijssen
Erasmus University Department:	Pedagogical Sciences
Research School:	Institute for the Study of Education and Human Development
Promotors:	Prof. dr. M. H. van IJzendoorn, Prof. dr. H. Tiemeier, Prof. dr. M. J. Bakermans-Kranenburg
Copromotor:	Dr. T. White

	Year	Workload (ECTS)
1. PhD training		
Courses		
NIHES Summer Program, Erasmus Medical Center, Rotterdam, the Netherlands	2011	
Principles of Research in Medicine		0.7
Methods of Clinical research		0.7
Cohort Studies		0.7
Social Epidemiology		0.7
The Practice of Epidemiologic Analysis		0.7
Academic English, Leiden University, Leiden, the Netherlands	2011	1
fMRI Data and Analysis, Leiden University, Leiden, the Netherlands	2012	5
FreeSurfer, Amsterdam, the Netherlands	2012	1.5
Attachment: State of the Art, Leiden University, Leiden, the Netherlands	2012	5
Applied Multivariate Data Analysis, Leiden University, Leiden, the Netherlands	2012-2013	10
NIHES Study design, Erasmus Medical Center, Rotterdam, the Netherlands	2013	4.3
Human Neuroanatomy to Psychopathology, Maastricht University, Maastricht, the Netherlands	2014	1.5
FSL, Oxford, United Kingdom	2014	1.5
Mortimer D. Sackler, M. D. Summer Institute, New York, New York, United States	2015	1

	Year	Workload (ECTS)
Seminars, workshops and symposia		
Generation R Research meetings, Erasmus Medical Center, Rotterdam, the Netherlands Oral presentation	2011-2015	1
Attachment colloquia, Department of Child and Family Studies, Leiden University, Leiden, the Netherlands (Oral presentation) Oral presentation	2011-2015	3
ISED Research Days, Utrecht, the Netherlands	2011	0.6
PhD Day, Erasmus University of Rotterdam, the Netherlands	2012	0.3
Lorentz Symposium on Ostracism, Exclusion and Rejection, Leiden, the Netherlands	2012	0.1
Symposium on Brain Development and Developmental Disorders, Utrecht, the Netherlands	2012	0.3
Masterclass Abuse and Neglect, Leiden University, Leiden, the Netherlands	2012	0.3
Nutrimenthe: Nutrition and Cognitive Function, Rotterdam, the Netherlands	2012	0.1
ISED Research Days, Utrecht, the Netherlands Oral presentation	2012	0.3
ISED Research days, Amsterdam, the Netherlands Oral presentation	2012	0.3
Understanding Each Other: Towards an Interdisciplinary Conceptualization of Empathy, Utrecht University, Utrecht, the Netherlands	2014	0.3
Sophia Research Days, Rotterdam, the Netherlands Poster presentation	2014	0.1
ISED Research Days, Amsterdam, the Netherlands Poster presentation	2014	0.3
CID symposium, Leiden, the Netherlands Oral presentation	2015	0.3

	Year	Workload (ECTS)
International conferences		
Human Brain Mapping, Hamburg, Germany Poster presentation	2014	1
SRCD Attachment Pre-Conference, Philadelphia, Pennsylvania, United States	2015	0.3
SRCD Biennial Meeting 2015, Philadelphia, Pennsylvania, United States Poster presentation	2015	1
International research projects		
MIND Research Network, Albuquerque, New Mexico, United States Project on functional connectivity and psychopathy/substance abuse in juvenile delinquents under supervision of Prof. dr. Kent A. Kiehl.	2015	
Scholarships and grants		
Vereeniging Trustfonds Erasmus Universiteit Rotterdam, travel grant	2015	
2. Teaching activities		
Supervising Master's and Bachelor's theses		
Elva Koenen, Leiden University, research internship *Project title:* Gender differences in children's cognitive performance	2012	1.5
Rebecca Doualla, Amsterdam University, Master's thesis *Project title:* The neural substrate of aggression: a study on cortical thickness in the ACC and OFC and child reported aggression	2013	1
Nuray Dogan, Pedagogical Sciences, Erasmus University of Rotterdam, Bachelor's thesis *Project title:* Opvoedingsstijlen met een risico: invloed van opvoedingsstijlen op antisociaal gedrag: een reviewstudie	2014	1.5
Daniek Gouw, Pedagogical Sciences, Erasmus University of Rotterdam, Bachelor's thesis, second reader *Project title:* Het effect van een E-Health psycho-educatiecursus bij ouders van jonge kinderen met autisme	2014	0.3

	Year	Workload (ECTS)
Qeren Anson, Pedagogical Sciences, Erasmus University of Rotterdam, Bachelor's thesis, second reader *Project title:* Een review studie naar het effect van kinderopvang op het sociaal emotioneel functioneren van kinderen van immigranten.	2014	0.5
Tutoring		
Introduction to Pedagogical Sciences, Pedagogical Sciences, Erasmus University of Rotterdam	2011	1.5
Statistics I, Pedagogical Sciences, Erasmus University of Rotterdam	2011	1.5
Alignment of Education and Upbringing, Pedagogical Sciences, Erasmus University of Rotterdam	2013	1.5
History of Education and Upbringing, Pedagogical Sciences, Erasmus University of Rotterdam	2014	1.5
Orthopedagogy: Child Disorders, Pedagogical Sciences, Erasmus University of Rotterdam	2014	1.5
Biological Determinants of Learning and Development, Pedagogical Sciences, Erasmus University of Rotterdam	2014	3.0
Neuropsychological Testing, Pedagogical Sciences, Erasmus University of Rotterdam	2014	2
Orthopedagogy: Diagnostics, Pedagogical Sciences, Erasmus University of Rotterdam	2014	1.5
Lecturing		
Lie-telling in Children, Pedagogical Sciences, Erasmus University of Rotterdam	2014	0.1

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