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REPORT



Socialization of prosocial behavior: Gender differences in the mediating role of child brain volume

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ABSTRACT

Evidence has been accumulating for the impact of normal variation in caregiving quality on brain morphology in children, but the question remains whether differences in brain volume related to early caregiving translate to behavioral implications. In this longitudinal population-based study ($N = 162$), moderated mediation was tested for the relation between parental sensitivity and child prosocial behavior via brain volume, in boys and girls. Both maternal and paternal sensitivity were repeatedly observed between 1 and 4 years of age. Brain volume was assessed using magnetic resonance imaging measurements at age 8, and self-reported prosocial behavior of children was assessed at 9 years of age. Parental sensitivity was positively related to child brain volume, and to child prosocial behavior at trend level. Child brain volume was negatively related to child prosocial behavior. A significant gender-by-brain interaction was found, illustrating that daughters of sensitive parents were more prosocial and that less prosocial behavior was reported for girls with a larger total brain volume. Child gender significantly moderated the indirect effect of parental sensitivity on prosocial behavior via total brain volume. A significant indirect pathway was found only in girls. The results warrant replication but indicate the importance of considering gender when studying the behavioral implications of differences in brain volume related to early caregiving experiences.

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Brain development is not only dependent on genetic factors, but environmental factors can also shape child brain development (Belsky & de Haan, 2011; Richards et al., 2016). Pioneering studies on the impact of the environment focused on extremely negative experiences, including maltreatment (Riem, Alink, Out, van IJzendoorn, & Bakermans-Kranenburg, 2015) or institutionalized care (Nelson, Fox, & Zeanah, 2014). Exposure to extreme early adversities was shown to be related to alterations in brain growth,

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although direction of findings varies across studies, with both increased and decreased volumes reported. Prolonged exposure to adversities was related to larger changes in brain volume. Subsequently, studies have illustrated that even normal variation in early caregiving quality, such as the variation in parental sensitivity, warmth, and support (Kok et al., 2015; Luby et al., 2012; Whittle et al., 2014), may be related to differences in brain volume and growth in childhood and adolescence.

Although experience-dependent brain development seems a replicable finding, there are inconsistencies in the direction of the effect (Richards et al., 2016; Whittle et al., 2014). Moreover, high heritability estimates for brain growth (Jansen, Mous, White, Posthuma, & Polderman, 2015) have also emerged and studies have illustrated the large degree of variability in brain volume and brain development trajectories in healthy children (Brain Development Cooperative Group, 2012; Lenroot & Giedd, 2006). Yet, variation in temporal gray and white matter and frontal white matter volume in a normative sample has been found to be related to differences in performal IQ (Lange, Froimowitz, Bigler, & Lainhart, 2010). The question remains whether individual differences in brain volume related to early caregiving actually translate into meaningful differences in child behavior. In this study, we examine the behavioral implications for prosocial behavior of a previously published longitudinal association between early childhood parental sensitivity and child brain volume at age 8 (Kok et al., 2015). We hereby follow the two-pronged advances as postulated by Belsky and De Haan (2011) for research on the role of parenting in brain development: (a) studying the implications of normal variation in parenting for brain development and (b) illuminating whether parenting effects translate to child behavior.

To the best of our knowledge, only one study has investigated whether structural brain parameters related to normal variation in caregiving are predictive of academic outcomes (Whittle et al., 2016). In this study, increases in cortical thickness of the right superior frontal cortex mediated the association between maternal aggression and adolescent's school noncompletion. However, maternal positive behavior was not related to brain structure. In the current study, we include a more comprehensive measure of positive parenting, i.e., repeated measures of sensitive parenting of both mothers and fathers in early childhood; and we focus on child prosocial behavior, as there is robust evidence for its association with parental positive socialization strategies (Hastings, Miller, & Troxel, 2015).

Method

The study was embedded within the Generation R Study, a prospective cohort investigating growth, development, and health from fetal life onwards in Rotterdam, the Netherlands (Jaddoe et al., 2010). Detailed measurements were obtained in a subgroup of Dutch national origin, the Generation R Focus Cohort, to reduce confounding and effect modification (e.g., Luijk et al., 2010). The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Magnetic resonance imaging (MRI) scans were only allowed for children over 6 years of age. Written informed consent was obtained from all adult participants.

From 2009 until 2013, 1070 six- to ten-year-old children from the Generation R Study were invited to participate in a MRI component of the study (White et al., 2013).

396 children of the Generation R Focus Cohort were invited to participate in the MRI component of the study

↓
80 parents were contacted but refused participation; 5 children could not participate due to contraindication for MRI; 5 families were not reached.

306 children had an appointment: the appointment contained a neuropsychological assessment, mock scan session and MRI session

↓
60 children did not participate in the MRI session, because they decided to only participate in the neuropsychological assessment or stopped during or after the mock scan session.

For **246** children a T1 scan was available.

↓
For 26 children the initial T1 scan was judged unusable or poor, or images could not be processed in FreeSurfer, or segmentation quality was poor.

For **220** children MRI quality was good to excellent.

↓
For 27 children parental sensitivity data was not available, because of refusal to participate in the 3 year laboratory visit and the 4 year home visit.

For **193** children at least one measure of parental sensitivity (1-4y) was available.

↓
One twin-pair was excluded, because brain growth is not comparable to singletons.

N=191

↓
For 29 children self-reported prosocial behavior was missing due to nonresponse.

For **162** children self-reported prosocial behavior (9y) was available.

Figure 1. Flow chart depicting the inclusion and exclusion criteria.

Of these, 396 children also participated in the Generation R Focus Cohort. All inclusion and exclusion steps are explained in Figure 1. In short, 90 children could not participate because parents refused participation, because of a contraindication for participation (i.e., motor or sensory disorder, head trauma with history of loss of consciousness, neurological condition, claustrophobia), or because they could not be reached. Moreover, for 60 children no MRI scan was available and for 26 children data was of insufficient quality, i.e., the initial T1 scans were judged unusable or poor, or images could not be processed in FreeSurfer, or segmentation quality was poor (see also White et al., 2013). For 220 children, the data was of sufficient quality. For 193 children, at least one measure of early childhood parental sensitivity was available. We excluded one twin pair, resulting in 191 dyads. For 162 of the 191 dyads, child-reported prosocial behavior at age 9 was available. A nonresponse analysis of the 31 parent-child dyads excluded from analyses indicated that they did not differ in gender, parental educational level and sensitivity, child brain volume, and prosocial behavior. Mothers of children included in the analyses were older than mothers of excluded children, $t(35) = -2.27$, $p < .05$. The sample consisted of 51.2% girls. Average scores on child prosocial behavior were 13.7 ($SD = 1.3$) and the average IQ was 107.2 ($SD = 13.6$). The mean age of the mother and father at intake was 32.1 ($SD = 3.3$) and 34.0 ($SD = 4.5$), respectively. Of the parents, 63% had a high educational level.

Parental sensitivity was observed when the children were 1, 3, and 4 years of age, during free play, a psychophysiological assessment, or during teaching tasks. Sensitivity was observed using the Ainsworth's nine-point rating scales for *Sensitivity* and *Cooperation* (Ainsworth, Bell, & Stayton, 1974) at 1 year and the revised Erickson seven-point rating scales for *Supportive presence* and *Intrusiveness* (Egeland, Erickson, Clemenhagen-Moon, Hiester, & Korfmacher, 1990) at 3 and 4 years. At 1 and 3 years of age, child and primary caregiver (respectively 86% and 82% mothers) were observed, at 4 years child participated with both parents (response rate: 91% mothers; 100% fathers). Inter-coder reliability (intraclass correlation coefficient, single measure, absolute agreement) varied between .65 and .84. Further details about the assessment of parental sensitivity have been reported elsewhere (Kok, Linting, et al., 2013; Kok, van IJzendoorn, et al., 2013; Lucassen et al., 2015). A composite sensitivity score was created by averaging the standardized scores on maternal and paternal sensitivity.

MRI was performed around 8 years of age ($M = 8.06$, $SD = 0.95$). Images were acquired on a 3 T scanner (750 Discovery, GE Healthcare, Milwaukee, WI) using an eight-channel head coil and a sagittal T₁ inversion recovery fast-spoiled gradient recalled sequence; TE = 4.24ms, T₁ = 350ms, TR = 10.26ms, NEX = 1, flip angle = 16°, and resolution 0.9 mm³ isotropic. Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite 5.1. The technical details of these procedures are described elsewhere (Reuter, Schmansky, Rosas, & Fischl, 2012). Briefly, processing included intensity normalization, removal of nonbrain tissue, automated Talairach transformation into standard space, and segmentation of the cortical and subcortical white/gray matter structures (Fischl & Dale, 2000). The following volumes were analyzed: total brain, gray matter, and white matter volume, as no evidence was found for an association between parental sensitivity and subcortical volumes (Kok et al., 2015). Volume measures were z-standardized to facilitate interpretation.

Prosocial behavior was assessed with the self-report version of the prosocial scale of the Strengths and Difficulties Questionnaire (Goodman, Meltzer, & Bailey, 1998; Muris, Meesters, & van den Berg, 2003). Children completed this questionnaire when they were approximately 9 years ($M = 9.68$ years, $SD = 0.26$). The scale consists of five items, e.g., “I am nice to other children,” scored on a three-point Likert scale (1 = *not true*, 2 = *somewhat true*, 3 = *certainly true*; $\alpha = 0.60$). Scale scores were square-root-transformed to approach normality and reversed for interpretation purposes.

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). Furthermore, analyses were controlled for the average parental educational level. If paternal educational level was missing ($n = 20$), maternal educational level was taken as an indicator of family educational level.

First, the bivariate associations between parental sensitivity (predictor), child brain volume (mediator), and prosocial behavior (outcome) were explored. Second, multiple regression analyses on the prediction of parental sensitivity and child brain volume (total, gray, white matter) for prosocial behavior were performed using SPSS 23 (IBM Corp., 2015), with 1,000 bootstrap samples using case resampling with replacement. The analyses were adjusted for child gender, age, and parental educational level. Moreover, interaction terms between child brain volume and gender were included in the model and computed after centering of the constituent variables. If interaction effects were significant, the sample was stratified by gender to investigate the associations between brain volume and prosocial behavior for boys and girls separately. A mediation model was tested with child brain volume as mediator of the association between parental sensitivity and child prosocial behavior, using PROCESS (Preacher & Hayes, 2008). The mediation model was run with 95% bias-corrected bootstrap confidence intervals applying 5,000 bootstrap samples using case resampling with replacement. In case of significant gender-by-brain interactions, moderated mediation was tested using PROCESS (Preacher & Hayes, 2008), with 95% bias-corrected bootstrap confidence intervals applying 5,000 bootstrap samples using case resampling with replacement. When the index of moderated mediation was significant, the mediation model was run for girls and boys separately. The false positive level for all analyses was $\alpha = 0.05$.

Results/discussion

Boys had a larger total brain, gray, and white matter volume than girls (all $p < .001$). Moreover, girls reported higher levels of prosocial behavior than boys, $t(160) = -4.06$, $p < .001$. Parents with a high educational level were more sensitive than parents with a low/medium educational level, $t(160) = -2.72$, $p < .01$. Children from a highly educated family had a larger total gray matter volume, $t(160) = -2.09$, $p < .05$.

Bivariate correlations indicated that parental sensitivity in early childhood was positively related to prosocial behavior at age 9 at trend level ($r = .15$, $p = .06$). The total brain and white matter volume of the child at age 8 were negatively

related to prosocial behavior at 9 years ($r = -.18, p < .05$; $r = -.20, p < .05$, respectively). As already reported in a previous study (Kok et al., 2015), parental sensitivity in early childhood was positively related to total brain, $r = .23, p < .01$, white matter, $r = .20, p < .01$, and gray matter volume, $r = .24, p < .01$, of children at age 8.

In multiple regression analysis, a significant gender-by-brain interaction was found for child prosocial behavior (see Table 1). Exploration of the predictive model in boys and girls separately demonstrated that daughters of more sensitive parents were more prosocial ($B = .17, 95\% \text{ CI} = [.02, .34]$) and that girls with a larger total brain volume were less prosocial ($B = -.10, 95\% \text{ CI} = [-.20, -.01]$) (see Table 1).

The effect of gender on the indirect effect of parental sensitivity on child prosocial behavior through child brain volume was significant ($B = -.05, 95\% \text{ CI} = [-.15, -.01]$). In girls, the mediation model demonstrated a significant indirect effect for parental sensitivity on prosocial behavior via total brain volume, $B = -.07, 95\% \text{ CI} = [-.16, -.01]$ (see Figure 2). For boys, mediation was not found, $B = .01, 95\% \text{ CI} = [-.01, .06]$. The analyses above were repeated for child gray and white matter volume and these analyses showed similar results (see Table 1). In girls, again, the mediation model demonstrated a significant indirect effect for parental sensitivity on prosocial behavior via white matter volume, $B = -.05, 95\% \text{ CI} = [-.15, -.01]$, and via gray matter volume, $B = -.06, 95\% \text{ CI} = [-.16, -.01]$.

Our findings illustrate that the association between sensitivity, child brain volume, and prosocial behavior is moderated by gender: for girls but not for boys, early childhood parental sensitivity predicts higher levels of prosocial behavior via brain volume. Although parental sensitivity was related to a larger total brain volume and higher levels of prosocial behavior in girls, a larger total brain volume was associated with less prosocial behavior. This pattern of inconsistent mediation (MacKinnon, Krull, & Lockwood, 2000) could be indicative of a suppression effect although confounding cannot be excluded. Studies on the association between brain volume parameters and prosocial behavior are scarce and not focused on global brain volume. In a recent study on the same data set, a negative association was found 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 covering the right superior parietal cortex, cuneus, and precuneus in girls (Thijssen et al., 2015). In a sample of very preterm children, the bifrontal diameter at term was positively associated with socio-emotional development in boys only (Rogers et al., 2012). Shdo et al. (in press) found associations between prosocial motivation and nucleus accumbens, caudate head, and inferior frontal gyrus, using a neurodegenerative disease lesion model. Our study is one of the small and increasing number of studies suggesting that differences in child brain volume in the general population, related to normal variation in parenting, can potentially translate to behavioral differences. However, due to the modest size of the (significant) mediation pathway, the small sample, and possible residual confounding by covariates not included, e.g., genetic factors, this result needs replication. The high degree of variability in brain volume in typically developing children highlights the need to be cautious in drawing conclusions about behavioral implications of brain volume variations (Brain Development Cooperative Group, 2012; Lenroot & Giedd, 2006). Interestingly, a recent study on parenting and adolescent brain structure found a mediating pathway showing

Table 1. Regression model predicting prosocial behavior.

	Children (N = 162)			Girls (n = 83)			Boys (n = 79)		
	B (SE)	p	R ²	B (SE)	p	R ²	B (SE)	p	R ²
			95% CI			95% CI			95% CI
TBV									
Step 1:	Gender	.19(.06)**	.10	-.	-.07(.09)	.07	-.07(.09)	-.07(.09)	.00
	Educ level parents	-.02(.05)		.03(.08)			-.07(.09)	-.07(.09)	
	Age at MRI	-.04(.03)		-.06(.04)			-.01(.05)	-.01(.05)	
Step 2:	Parental sens (1-4yrs)	.13(.06)*	.13	.17(.08)*	.043	.18	.08(.10)	.08(.10)	.03
	Total brain vol. (8y)	.04(.05)		-.10(.05)*	.036		.04(.05)	.04(.05)	
Step 3:	Gender*Total brain vol.	-.14(.07)*	.17	-.10(.05)*			-.04(.05)	-.04(.05)	
WHITE	Gender	.18(.06)**	.10	-.03(.07)		.07	-.06(.09)	-.06(.09)	.00
Step 1:	Educ level parents	-.02(.06)		-.06(.04)			-.01(.05)	-.01(.05)	
	Age at MRI	-.04(.03)		.16(.09)			.08(.10)	.08(.10)	
Step 2:	Parental sens (1-4yrs)	.12(.07)	.13	-.10(.05)*	.034	.17	.04(.05)	.04(.05)	.03
	White matt vol. (8y)	.04(.05)		-.10(.05)*			-.06(.09)	-.06(.09)	
Step 3:	Gender*White matt vol.	-.14(.06)*	.17	-.10(.05)*			-.06(.09)	-.06(.09)	
GRAY	Gender	.19(.06)**	.10	-.03(.07)		.07	-.07(.09)	-.07(.09)	.00
Step 1:	Educ level parents	-.02(.06)		-.06(.04)			.00(.04)	.00(.04)	
	Age at MRI	-.04(.03)		.16(.09)			.08(.10)	.08(.10)	
Step 2:	Parental sens (1-4yrs)	.13(.06)*	.13	-.10(.05)*		.16	.08(.10)	.08(.10)	.03
	Gray matt vol. (8y)	.03(.04)		-.09(.05)			.04(.05)	.04(.05)	
Step 3:	Gender*Gray matt vol.	-.13(.07)	.13	-.09(.05)			-.04(.05)	-.04(.05)	

B and CI are based on the fully adjusted models and 1,000 bootstrap samples.

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

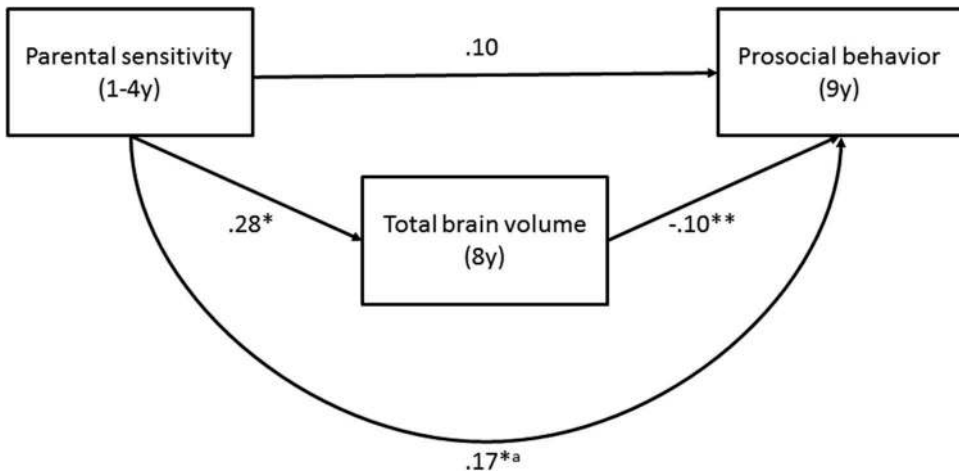


Figure 2. Total brain volume mediates the association between parental sensitivity and prosocial behavior in girls.

^aIndirect path * $p < .05$; ** $p < .01$.

that changes in brain structure related to observed maternal aggression were predictive of adolescent's school noncompletion (Whittle et al., 2016). No mediation for positive parenting was found, but in the study by Whittle et al. positive parenting consisted of maternal caring/positive/neutral affect, whereas in our study the broader concept of sensitivity was captured, defined by prompt and adequate response of *both* parents to the child's signals.

Our study underlines gender differences in prosocial behavior and its predictors, as for boys, early childhood parental sensitivity and brain volume at age 8 did not predict prosocial behavior at age 9. The fact that variance in all pertinent variables was equal for boys and girls makes a purely statistical explanation for the gender moderation less plausible. It has been suggested that measures of prosocial behavior can be gender-biased, including more "feminine" aspects, e.g., empathy and sympathy, as compared to more "masculine" aspects, e.g., engagement and active prosocial behavior (Hastings et al., 2015). Perhaps for boys, these unmeasured masculine elements of prosocial behavior are more relevant and subject to parental influence and related to brain volume.

Overall, this study illustrates the importance of taking into account gender in studying behavioral implications of differences in child brain volume. Moreover, the findings suggest that differences in brain volume related to normal variation in early childhood parental sensitivity may potentially translate into variations in children's prosocial development.

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