The social brain in middle childhood: a neurobiological perspective on individual differences in social competence

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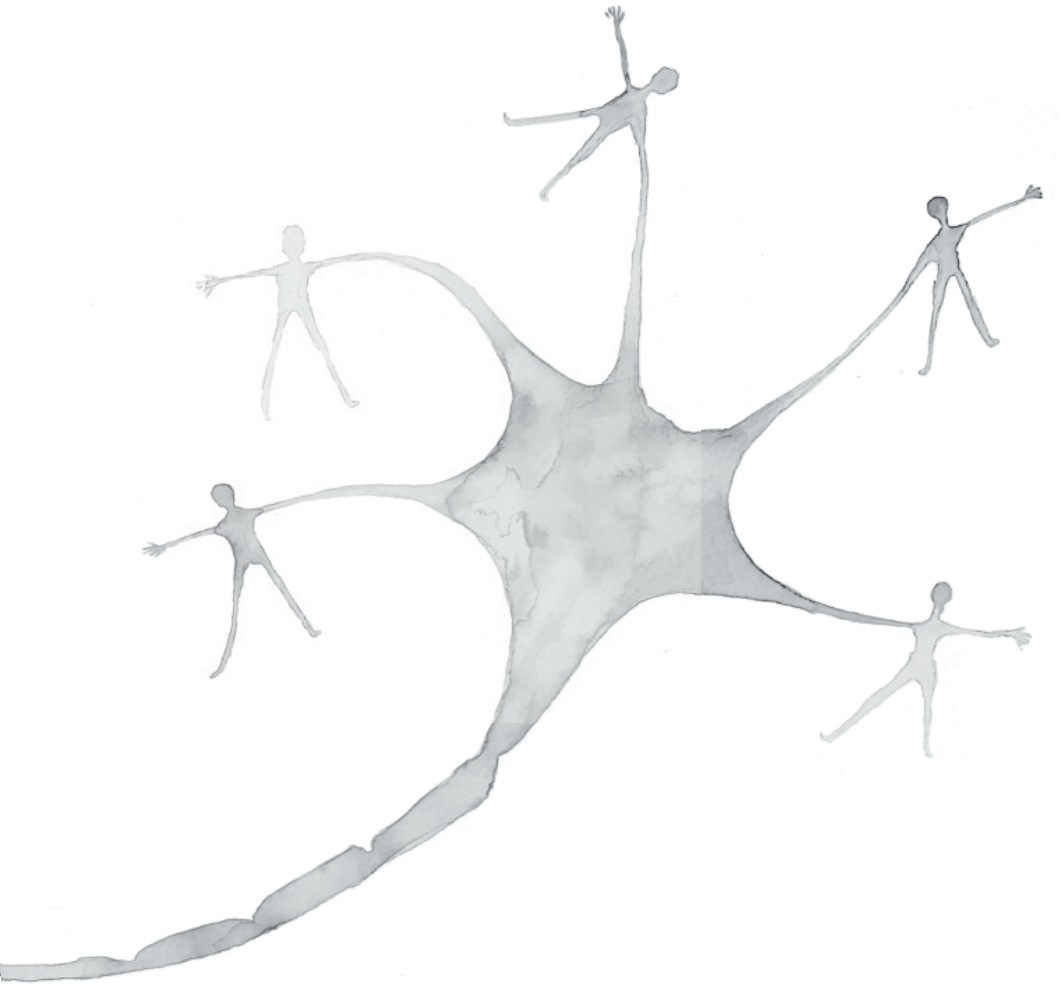
CHAPTER 5

Genetic and environmental influences on structure of the social brain in childhood



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Abstract

Prosocial behavior is an important aspect of developing social relations in childhood. Prior studies showed protracted structural development of social brain regions associated with prosocial behavior. However, it remains unknown to what extent structure of the social brain is driven by genetic or environmental influences, and whether similar genetic factors explain variance in structure of the social brain and prosocial behavior. The current study examined this hypothesis in a twin sample (aged 7-9-year; n = 486). Surface area and cortical thickness of the medial prefrontal cortex (mPFC), precuneus, temporo-parietal junction (TPJ) and posterior superior temporal sulcus (pSTS) were analyzed. Results showed genetic contributions to surface area and cortical thickness for all brain regions. We found additional shared environmental influences for TPJ, suggesting that this region is relatively more sensitive to social experiences. We also found strong evidence of genetic influences on parent-reported prosocial and empathic behavior. The precuneus shared genetically determined variance with empathic behavior, suggesting a genetic overlap in brain structure and empathic prosocial behavior. These findings show that both structure of the social brain and empathic prosocial behavior are driven by a combination of genetic and unique environmental factors, with some factors overlapping for brain structure and behavior.

Introduction

Developing and maintaining social relations with others is often dependent on prosocial behavior, which can be defined as voluntary behaviors to benefit another individual (e.g. helping and sharing; (Eisenberg et al., 2006). Many prior studies have investigated the origins of prosocial behavior in children and adolescents, using multiple indices such as self-report (van de Groep, Meuwese, Zanolie, Güroğlu, & Crone, 2018; Vrijhof et al., 2016), parent-report (Knafo-Noam et al., 2015; Thijssen et al., 2015), and experimental measures (Fehr et al., 2008). These studies showed that the first signs of prosocial behavior are already apparent in 18-month old children (Warneken & Tomasello, 2006), but at the same time this behavior continues to develop over childhood and adolescence (Eisenberg et al., 2006; Güroğlu et al., 2014). This leads to the question whether prosocial behavior is inherently present or whether this behavior is learned through social experiences (Blakemore & Mills, 2014).

One approach to investigate the factors that may contribute to prosocial behavior is by examining the neural processes that underlie social behaviors. Recently, researchers have demonstrated a distinct set of brain regions (known as the "social brain") that are recruited during (pro)social thoughts and actions using functional neuroimaging, including the medial prefrontal cortex (mPFC), temporal parietal junction (TPJ; Blakemore, 2008; Burnett & Blakemore, 2009; Gunther Moor et al., 2012; Will et al., 2015), posterior superior temporal sulcus (pSTS; Blakemore, 2008; Frith & Frith, 2003), and precuneus (Carrington & Bailey, 2009). Interestingly, at the structural level these brain regions continue to develop throughout childhood and adolescence (Mills et al., 2014), but it is currently unknown to what extent the development of these regions is biologically programmed or sensitive to environmental influences. Also, no study to date examined the genetic and environmental influences on the social brain in relation to prosocial behavior.

This question can be examined in more detail by using a twin design that allows for distinguishing between genetic and environmental influences. By comparing behaviors of monozygotic twins (who share 100% of their genes) with dizygotic twins (who share on average 50% of their genes), it is possible to unravel whether processes are more strongly driven by additive genetic factors, shared environment (family-related factors), or unique

environment (child-specific factors; McLoughlin et al., 2007; Plomin et al., 2001). Prior studies using this approach showed that overall brain volume is strongly sensitive to genetic effects (Peper et al., 2007; Teeuw et al., 2018) but it is not yet known whether this is different for regions in the social brain. These regions are of specific interest, given that they support social behaviors, and therefore may be more open to environmental and social experiences (Blakemore & Mills, 2014). A prior study by Mills et al. (2014) distinguished between three indices of structural development: cortical thickness, surface area and cortical volume (the latter being the product of thickness and surface area) and focused on the key regions in the social brain typically involved in social behavior (Blakemore, 2012). They showed that cortical volume of the mPFC, TPJ, and pSTS follows a cubic trajectory, peaking around age 9. In contrast, cortical thickness showed linear decreases across development, whereas surface area shows a cubic trajectory, similar to cortical volume, with different peaks for mPFC (around age 8), TPJ (around age 11), and pSTS (around age 13). These findings converge with prior studies showing that total cortical thickness and surface area have distinct developmental patterns (Gilmore, Knickmeyer, & Gao, 2018; Raznahan et al., 2011; Tamnes et al., 2017; Vijayakumar et al., 2016; Wierenga et al., 2014).

In the current study we advance these findings by examining genetic and environmental influences on measures of surface area and cortical thickness of key regions in the social brain: the mPFC, TPJ, pSTS, and precuneus. We specifically focus on middle childhood as this is a transition period to the pronounced grey matter changes of adolescence (Mills et al., 2016; Wierenga et al., 2014). Prior studies showed that surface area is more susceptible to varying environmental influences than cortical thickness (Noble et al., 2015), which in turn is consistent with the finding that surface area growth showed more individual differences than cortical thickness growth (Mills et al., 2014). It should be noted that some other studies demonstrated that shared environmental influences, such as SES, are larger for changes in cortical thickness rather than changes in surface area (Piccolo et al., 2016). It therefore remains an unanswered question which measure of brain structure is more sensitive to environmental influences.

Heritability studies on prosocial behavior revealed that prosocial behavior as indicated by parent-report is strongly influenced by genetics in children at the age of seven years, with heritability estimates ranging from 60-69% (Knafo-Noam et al., 2015; Knafo &

Plomin, 2006). An experimental study using a prosocial compensation task in 7-9-year-old children, however, did not find significant genetic or shared environmental influences (van der Meulen, Steinbeis, Achterberg, van Ijzendoorn, & Crone, 2018). Nevertheless, studies with adolescents showed that prosocial behavior is sensitive to peer pressure, suggesting that the environment can also impact prosocial behavior (Foulkes, Leung, Fuhrmann, Knoll, & Blakemore, 2018). Possibly, these different findings are due to use of different methods to measure prosocial behavior, with parent-report measuring prosocial behavior across contexts, and experimental tasks measuring prosocial behavior in a specific situation. Furthermore, different subcomponents of prosocial behavior such as empathy (an emotional reaction that is elicited by another individual's emotional response) and perspective taking (the ability to understand and perceive the motives, ideas, and wishes of others; Penner & Finkelstein, 1998) should be taken into account when estimating heritability. For example, Knafo, Zahn-Waxler, Van Hulle, Robinson, and Rhee (2008) showed that empathy and prosocial behavior share genetic and unique environmental influences in childhood. Therefore, in this study we focused on parent-reported prosocial behavior and empathy in relation to structural estimates of the social brain.

So far there is little understanding of the underlying biological processes driving prosocial behavior in children, and only two cross-sectional studies have focused on associations between brain structure and prosocial behavior in children. Wildeboer et al. (2018) found a positive association between cortical thickness of the pars orbitalis and pre- and post-central cortex and costly donating behavior in 8-year-old children. In addition, Thijssen et al. (2015) found positive associations between cortical thickness of the mPFC and precuneus and parent-reported prosocial behavior in a large sample of 6-9-year-old children. To elaborate on these initial brain-behavior associations in children, we used the novel approach of simultaneously investigating unique as well as shared genetic and environmental influences on structure of the social brain and prosocial behavior in middle childhood.

Taken together, in the current study we investigated heritability of prosocial behavior and structure of the social brain (mPFC, TPJ, pSTS and precuneus) in a large middle childhood twin sample (N = 512, aged 7-9). First, we examined the extent to which variance in both prosocial behavior and structure of the social brain was accounted for by genetics,

shared or unique environment (Knafo-Noam et al., 2015; Panizzon et al., 2009). Within the structural measures of the social brain, we examined estimates of heritability for cortical thickness and surface area separately (Winkler et al., 2010). Second, we explored whether covariance in prosocial behavior and structure of the social brain was accounted for by overlapping genetic factors. Finally, we studied whether covariance in different components of prosocial behavior was accounted for by overlapping genetic factors in middle childhood (Knafo et al., 2008).

Methods

Participants

Participants were recruited for the longitudinal twin study of the Leiden Consortium on Individual Development (L-CID; Euser et al., 2016). We obtained address information through municipal registries and invited families with twin children (born between 2006-2008) to participate. Same-sex twin pairs were included in the study when they were 7-9 years old at the time of data collection, had normal (or corrected to normal) vision, were fluent in Dutch or English, and did not suffer from psychological or physical conditions that could hinder their performance on the tasks. The study was approved by the Dutch Central Committee on Research Involving Human Subjects (CCMO) and parental informed consent was obtained before data collection. Parents received financial compensation (€80) for their time invested in the study and children received a small gift.

We initially included 512 participants (256 same-sex twin pairs) in the L-CID study (previously described in Achterberg et al. (2018) and van der Meulen et al. (2018)). This population sample included 10 participants diagnosed with an Axis-I disorder (see Figure 1 for detailed information). Estimated participant IQ was within normal range (72.5 - 137.5; estimated via the subscales Block Design and Similarities of the Wechsler Intelligence Scale for Children, 3rd version (WISC-III; Wechsler, 1991). Twin zygosity was assessed using DNA information from buccal cell samples, collected via mouth swabs. Missing DNA information for one family was imputed with zygosity estimates derived from the Zygosity Diagnosis

Questionnaire (Rietveld et al., 2000). An overview of the participants included in analyses at various stages of the study can be found in Figure 1.

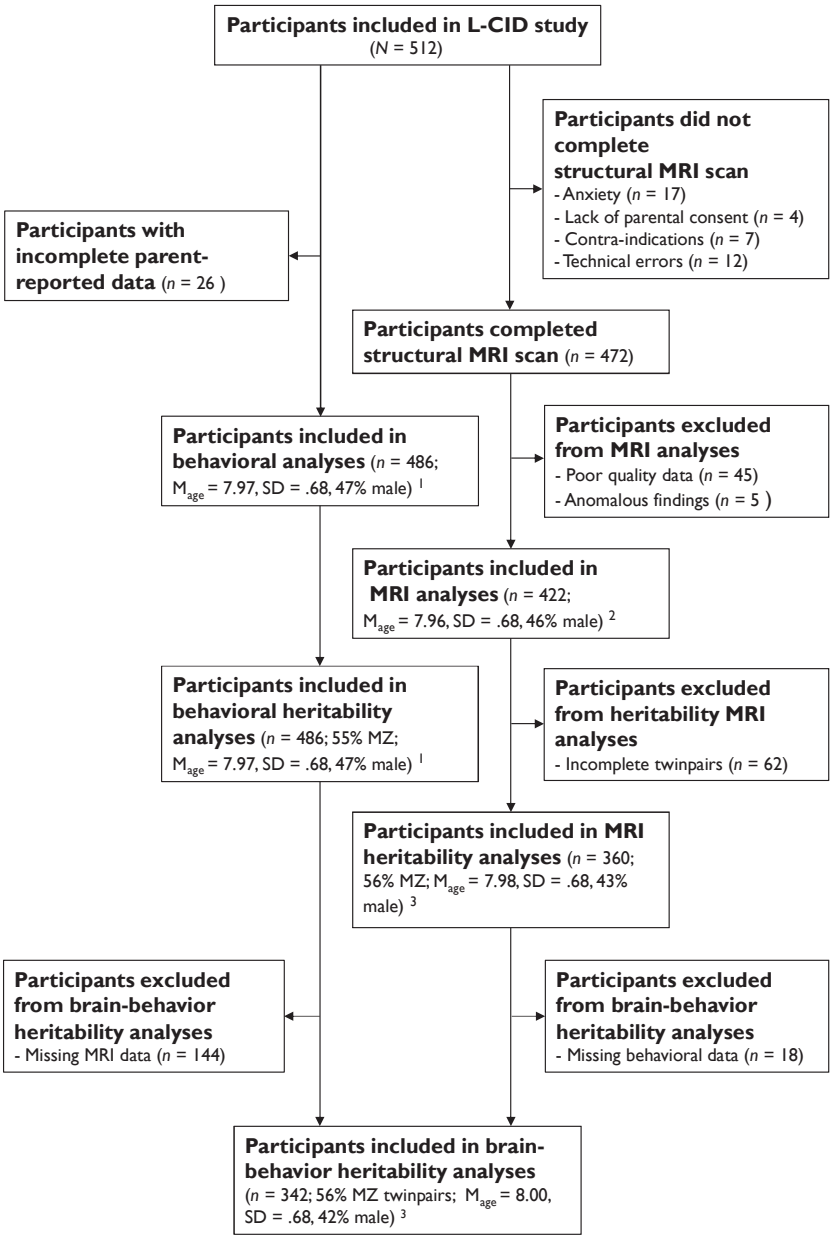


Figure 1. *Flowchart of inclusion of samples (including demographic information) at various stages of the study. MZ = monozygotic twin pairs; [1] Diagnosed Axis-I disorders: ADHD and/or ADD (eight participants), PDD-NOS (one participant), generalized anxiety disorder (GAD; one participant); [2] Diagnosed Axis-I disorders: ADHD and/or ADD (six participants), PDD-NOS (one participant), GAD (one participant); [3] Diagnosed Axis-I disorders: ADHD and/or ADD (five participants), PDD-NOS (one participant), GAD (one participant)*

Procedure

Both parents were asked to fill out several questionnaires before the lab visit. During the lab visit, participants were thoroughly prepared for the MRI procedure, by receiving extensive explanations and a practice session in a mock scanner. Co-twins were randomly assigned to either start with the scanning session or to start with other behavioral measures. During the scanning session, participants first completed a social evaluation fMRI task (Social Network Aggression Task; (Achterberg et al., 2018), and a prosocial compensating fMRI task (Prosocial Cyberball Game; (van der Meulen et al., 2018). After the fMRI tasks, a high resolution structural scan, DTI scans, and a resting state scan were collected.

MRI data acquisition and processing

MRI scans were acquired on a Philips Ingenia MR 3.0 Tesla scanner at the Leiden University Medical Center, using a standard 32-channel whole-head coil. A high resolution 3D T1-weighted anatomical image was collected (TR = 9.8 ms, TE = 4.6 ms, 140 slices, voxel size = 1.17 × 1.17 × 1.2 mm, and FOV = 224 × 177 × 168 mm). In order to reduce motion artifacts, foam inserts were used within the head coil to restrict head movement. In addition, participants were instructed to watch a child-appropriate movie during the T1-weighted scan acquisition in order to decrease head motion (Greene et al., 2018). Furthermore, to increase scan quality T1-weighted scans were visually inspected on motion artifacts during the scanning session (i.e. visible movement rings) and repeated if motion was detected (6% of participants).

Next, T1-weighted images without anomalous findings were processed in FreeSurfer (v5.3.0). Tissue classification and anatomical labeling was performed using the well-validated and well-documented FreeSurfer v5.3.0 software

(http://surfer.nmr.mgh.harvard.edu/). In short, this software includes non-brain tissue removal (Clarkson et al., 2011; Ségonne et al., 2004), segmentation of deep gray matter (Fischl et al., 2004; Fischl et al., 2004; Hutton, Draganski, Ashburner, & Weiskopf, 2009; Salat et al., 2004), intensity normalization (Sled, Zijdenbos, & Evans, 1998), and correction of gray-white matter boundary topology (Fischl, Liu, & Dale, 2001; Segonne, Pacheco, & Fischl, 2007).

For three of the regions of interest (mPFC, TPJ and pSTS; see Figure 2), we used a template based on Mills et al. (2014) for each T1-weighted scan. Note that we did not include the anterior temporal cortex (included as another region of interest in the study by Mills et al. (2014)) as cortical reconstruction of this region was unsuccessful for one or both hemispheres in a large number of participants (45% of sample). Additionally, the precuneus was derived from the Desikan-Killiany atlas (Desikan et al., 2006). For each labeled structure, we extracted measurements of surface area (in mm^2) and cortical thickness (in mm) for left and right hemisphere separately. As we did not hypothesize lateralization effects, we combined structural measures for each hemisphere. As such we had one value for surface area and one value for cortical thickness for each ROI. To compute bilateral measurements of surface area we averaged measurements for left hemisphere (lh) and right hemisphere (rh) surface area (SA):

$$(lh\ CT * lh\ SA) / 2$$

To compute bilateral measurements of average cortical thickness (CT), we took the size of each ROI into account (also see Bos et al. (2018)) by using the following formula:

$$\frac{(lh\ CT * lh\ SA) + (rh\ CT * rh\ SA)}{(lh\ SA + rh\ SA)}$$

Quality control of T1-weighted scans

To establish the quality of the T1-weighted scans that were collected, we manually rated whether the anatomical labeling was correct for each FreeSurfer pre-processed scan. Three raters (S.P, L.W., and M.M.) were trained to perform manual quality control (see Klapwijk,

van de Kamp, van der Meulen, Peters, and Wierenga (2019) for a detailed description of this procedure), using 20 scans from an independent dataset. Based on 39 scans from the current dataset, rated by all three raters, inter-rater reliability was sufficient (*ICC* = .55, $p < .05$). Out of 467 scans, 422 scans were rated to be of sufficient quality. Due to poor quality the other 45 scans (9%) were excluded from further analyses.

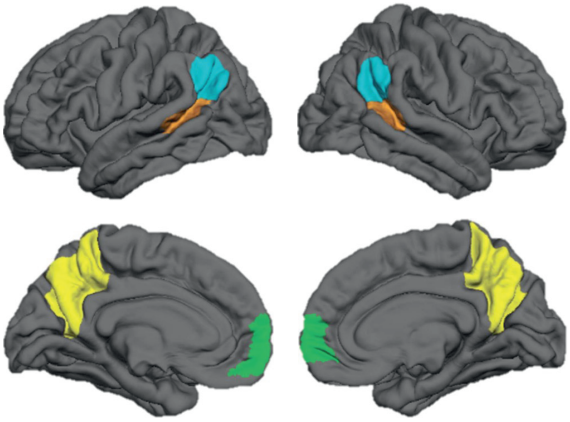


Figure 2. *Regions of interest in the social brain, including TPJ (blue), pSTS (orange), mPFC (green), and precuneus (yellow). Left side of the panel indicates left hemisphere, right side of the panel indicates right hemisphere.*

Parent-reported prosocial behavior

To measure parent-reported prosocial behavior we used subscales of two different questionnaires: the 5-item "Prosocial" subscale of the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997), and the 13-item "Empathic and Prosocial Response to Another's Distress" subscale of the My Child Questionnaire (MC; Kochanska, DeVet, Goldman, Murray, & Putnam, 1994). The SDQ subscale was answered with a 3-point Likert scale (1 = not true, to 3 = certainly true), and included items such as *"My child is considerate of other people's feelings"*. The MC subscale was answered with a 5-point Likert scale (1 = not true, to 5 = true) and included items such as *"My child will try to comfort or reassure another in distress"*.

The 18 items were factor analyzed using principal component analysis with Varimax rotation. To prevent within-twin dependence in the PCA, we randomly divided co-twins over two samples (A and B), such that one co-twin of each twin-pair was allocated to sample A and the other twin was allocated to sample B. Scores on the SDQ were recoded (from 1-2-3 to 1-3-5) in order to create a scale comparable to the scores on the MC (range 1-5). First, we ran the PCA on the items answered by one of the parents in sample A. KMO (.81) and Bartlett's test (X^2 (153) = 1185.21, $p < .001$) indicated that the 18 items were suitable for PCA. Our analysis yielded two factors. The first factor (explaining 26.15% of the variance) was labeled 'Prosocial' and had high loadings for items such as *"My child shares readily with other children"*. The second factor (explaining 13.11% of the variance) was labeled 'Empathy' and had high loadings for items such as *"My child is upset by stories in which characters are hurt or die"*. Two items did not fit well with either of the two components: *"My child may occasionally tease a pet if unsupervised"* (recoded) and *"My child feels good when good things happen to movie characters"*. These items were not included in further analyses (see Table S1 in the Appendices for an overview of the final subscale composition). We found a similar component structures with the other parent in sample A, and for both parents in sample B, indicating that this outcome was fitting for all participants and parents in our sample. Subscale scores were calculated by computing the mean of the items. We found positive correlations between both parents on the subscale 'Prosocial Behavior' (sample A: $r = .48$; sample B: $r = .53$, p's $< .001$) and 'Empathy' (sample A: $r = .37$; sample B: $r = .43$, p's $< .001$). Therefore, we created two new variables by calculating the mean rating of both parents for the subscale 'Prosocial Behavior' and for the subscale 'Empathy'. For both subscales, a higher score indicated more prosocial behavior or empathy.

Data analysis

Analyses were performed in SPSS (version 23.0; IBM SPSS Statistics, IBM Corporation) and R (version 3.3.2; R Core Team, 2015). Outliers detected in parent-reported prosocial behavior, surface area of mPFC and cortical thickness of TPJ were winsorized (Tabachnick & Fidell, 2013). Non-normally distributed variables (surface area of TPJ, pSTS, and precuneus) were log transformed. For parent-reported prosocial behavior and cortical thickness of TPJ

log transformation did not improve normality, so the untransformed variables were used in further analyses. Finally, in order to take into account effects of age, sex, and IQ on prosocial behavior and surface area and cortical thickness of the social brain, we performed regression analyses on all outcome measures, with age, sex, and IQ as predictor variables. We then used the unstandardized residuals as variables in our subsequent analyses.

To test heritability estimates for structural properties of the social brain and components of prosocial behavior we first computed within-twin pair Pearson correlations for each outcome variable, separately for MZ (monozygotic) and DZ (dizygotic) twins. For non-normal data we computed within-twin pair Spearman rank correlations. Since MZ twins share 100% of their genes, and DZ twins only share around 50% of their genes, a high MZ correlation would indicate influence of genetic factors. A DZ correlation higher than half the MZ correlation would indicate influence of shared environment (Knafo-Noam et al., 2015). MZ and DZ within-twin correlations coefficients smaller than 1 indicate additional effects of (unique) environment. We next computed univariate ACE models to inspect the relative contribution of genetic (A), shared environmental (C), and unique environmental factors and/or measurement error (E) to variance in brain structure and prosocial behavior, using the OpenMx package (version 2.7.4; Neale et al., 2016) in R. For each outcome variable, four different models (ACE, AE, CE, and E) were estimated. The fit of each model was then compared to the fit of a more parsimonious model (e.g. ACE to AE) by subtracting the -2 log likelihood (-2LL), resulting in an estimate of the Log-Likelihood Ratio Test (LRT). The LRT follows the X^2 distribution. The model with the least number of parameters that did not fit significantly worse than the more complex model (as indicated by LRT<3.84) was selected as the best fit. For models with equal numbers of parameters (i.e. AE and CE) the model with the lowest Akaike Information Criterion (AIC; Akaike, 1974) was selected.

To investigate shared heritability estimates we first inspected brain-behavior associations using least square regressions with brain structure predicting prosocial behavior. In order to overcome the nested nature of twin data, we used heteroscedasticity-consistent standard error (HSCE) estimations from the HSCE macro (Hayes & Cai, 2007), using the HC3 method (Ervin & Long, 2000). Using the same heteroscedasticity-correcting method, we also tested the association between prosocial behavior and empathy to further investigate shared heritability estimates for the components of prosocial behavior. Results

were Bonferroni-corrected for multiple testing, using a lowered threshold of $\alpha = .003$ for the 17 associations ($\alpha=0.05/17$).

Finally, we used bivariate ACE models to test the relative contribution of genetic (A), shared environmental (C), and unique environmental factors/measurement error (E) to covariance between measures of social brain structure and components of prosocial behavior, using the OpenMx package (version 2.7.4; Neale et al., 2016) in R. We performed a bivariate Cholesky decomposition model (see Figure 3), a base model for bivariate analyses (Neale & Cardon, 1992; Verweij, Mosing, Zietsch, & Medland, 2012). First a saturated Cholesky model was estimated, and next ACE, AE, CE, and E models were estimated. Similar to the univariate ACE models, the fit of each model was then compared to the fit of a less complex model (e.g. ACE to AE) using the LRT and AIC. After selecting the best fitting model, standardized path loadings were computed and squared to estimate the relative contribution of A, C, and E on variance in brain structure and prosocial behavior. These estimates are comparable to the estimates from the univariate ACE model. Next, we used the bivariate ACE model to estimate contributions of genes, shared and unique environment/measurement error to covariance (r_p) between brain structure and prosocial behavior (Plomin et al., 2001). The contribution of genes to the covariance was computed with the following formula:

$$\frac{\textit{estimate path } a11 * \textit{estimate path } a12}{\textit{covariance}}$$

using the standardized path loadings (Treur, Boomsma, Ligthart, Willemsen, & Vink, 2016). Contributions of shared and unique environment/measurement error to covariance were computed using the path loadings for paths *c* and e, respectively. Finally, we calculated the genetic (r_g) and environmental correlations (r_c and r_e) to quantify the extent to which brain structure and prosocial behavior are influenced by overlapping genetic and environmental factors. It should be noted that the heritability of both brain structure and prosocial behavior could be high, but the genetic correlation between them could be low, indicating that different genetic factors influence brain structure and prosocial behavior.

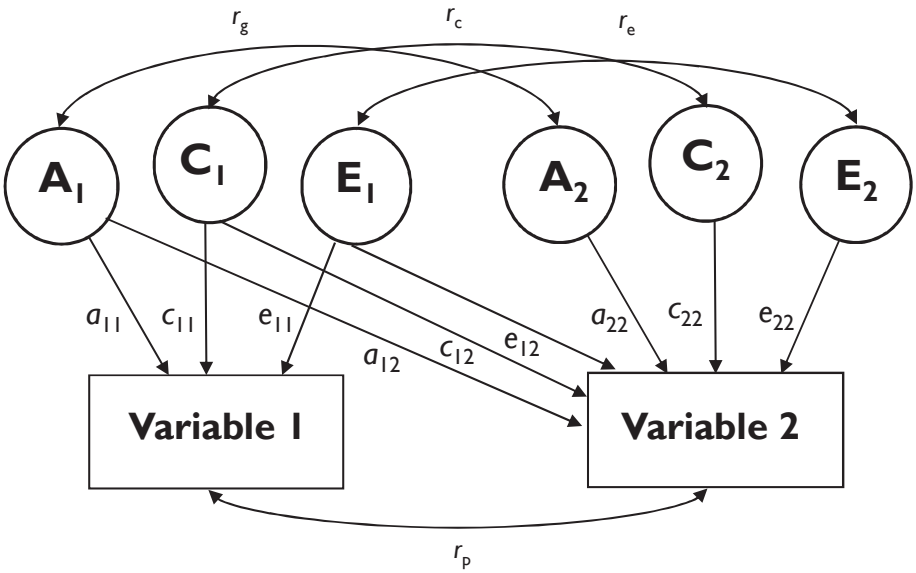


Figure 3. *Bivariate ACE model, visualizing contributions of genetic (A), shared environmental (C) and unique environmental (E) on two variables. Paths a_{11}-e_{11} and a_{22}-e_{22} indicate contributions of genes and environment on variables 1 and 2, respectively. Paths a_{12}-e_{12} indicate contributions of the factors for variable 1 to variable 2. Squaring standardized path loadings provide relative contributions of genetic and environmental factors. r_g = genetic correlation, r_c = shared environmental correlation, r_e = unique environmental correlation, r_p = covariance.*

Results

Univariate heritability of brain structure and prosocial behavior

First we addressed to what extent genetic, shared environment and unique environmental factors contribute to variation in surface area and cortical thickness of regions in the social brain and components of prosocial behavior.

For brain structure we examined genetic, shared environment and unique environment contributions for surface area and cortical thickness of mPFC, pSTS, TPJ, and precuneus separately. For surface area, within-twin correlations revealed significantly higher MZ than DZ concurrence for mPFC (r_{mz} = .50, r_{dz} = .20, Z = 2.27, p < .05), pSTS (r_{mz} = .63,

r_{dz} = .39, Z = 2.16, p < .05), and precuneus (r_{mz} = .82, r_{dz} = .29, Z = 5.63, p < .001), also see Table 1 and Figure S1. Within-twin correlations were comparable for TPJ (r_{mz} = .43, r_{dz} = .37, Z = .47, p > .05). We found substantial contributions of genetic factors for mPFC (A = 50%), pSTS (A = 51%) and precuneus (A = 81%), as well as a small contribution of shared environmental factors for pSTS (C = 12%). The remaining variance was best explained by unique environment/measurement error. Submodel fitting indicated that an AE model was best fitting for surface area of mPFC, pSTS and precuneus (see Table S2). For surface area of TPJ, both genetic factors (A = 24%) and shared environmental factors (C = 23%) contributed, and submodel fitting indicated that no clear distinction could be made between an AE and CE model. However, the confidence interval of the E factor (39-70%) in the full ACE model did not reach up to 100%, so it is likely that familial influences are present.

With respect to cortical thickness, within-twin correlations revealed significantly higher MZ than DZ concurrence for pSTS (r_{mz} = .36, r_{dz} = .12, Z = 1.68, p < .05) and precuneus (r_{mz} = .55, r_{dz} = .19, Z = 2.79, p < .005). Within-twin correlations for MZ and DZ twins were comparable for mPFC (r_{mz} = .23, r_{dz} = .15, Z = .54, p > .05) and TPJ (r_{mz} = .29, r_{dz} = .24, Z = .35, p > .05; see Figure S1). Estimations for contributions of genetics, shared environment and unique environment showed a substantial contribution of genetics for precuneus (A = 55%), and the remaining variance was best explained by unique environment/measurement error. Submodel fitting indicated that an AE model was best fitting for cortical thickness of precuneus (see Table S2). For cortical thickness of mPFC, TPJ, and pSTS both genetic (A = 16%, 8%, and 27%, respectively) and shared environmental factors (C = 6%, 22%, and 2%, respectively) contributed, and submodel fitting indicated that no clear distinction could be made between an AE and CE model. The confidence interval of the E factor did not include 100% however, providing room for familial influences.

Finally, within-twin correlations revealed significantly higher MZ than DZ concurrence for parent-reported prosocial behavior (r_{mz} = .37, r_{dz} = .05, Z = 2.59, p < .01) and empathy (r_{mz} = .76, r_{dz} = .41, Z = 4.29, p < .001). We found substantial contributions of genetics for prosocial behavior (A = 45%) and empathy (A = 62%), in addition to a smaller contribution of shared environment to empathy (C = 12%). The remaining variance was best explained by unique environment/measurement error. Submodel fitting indicated that an AE model was best fitting for both prosocial behavior and empathy (see Table S2).

Table 1. Within-twin correlations and estimated contributions of genes (A), shared environment (C), and unique environment/measurement error (E). 95% confidence intervals for each estimate are provided between parentheses.

Outcome variable	*r*MZ	*r*DZ	A^2	C^2	E^2	Best fitting model
Surface area						
mPFC	.50***	.20	0.50 (0.34-0.63)	0.00 ([‡]-0.25)	0.50 (0.37-0.66)	AE
TPJ	.43***	.37**	0.24 (0.00-0.60)	0.23 ([‡]-0.51)	0.53 (0.39-0.70)	AE/CE
pSTS	.63***	.39***	0.51 (0.14-0.73)	0.12 (0.00-0.45)	0.36 (0.27-0.49)	AE
Precuneus	.82***	.29*	0.81 (0.59-0.86)	0.00 ([‡]-0.21)	0.19 (0.14-0.27)	AE
Cortical thickness						
mPFC	.23*	.15	0.16 (0.00-0.39)	0.06 (0-0.31)	0.78 (0.61-0.96)	AE/CE
TPJ [†]	.29**	.24*	0.08 (0.00-0.47)	0.22 (0-0.41)	0.70 (0.53-0.86)	AE/CE
pSTS	.36***	.12	0.27 (0.00-0.43)	0.02 (0-0.36)	0.72 (0.57-0.88)	AE/CE
Precuneus	.55***	.19	0.55 (0.29-0.68)	0 (0.00-0.19)	0.45 (0.32-0.60)	AE
Parent report						
Prosocial behavior [†]	.37***	.05	0.45 (0.26-0.57)	0.00 (0.00-0.13)	0.55 (0.43-0.70)	AE
Empathy	.76***	.41***	0.62 (0.33-0.80)	0.12 (0.00-0.39)	0.26 (0.20-0.34)	AE

*r_{MZ} = within-twin correlation for monozygotic twins, r_{DZ} = within-twin correlation for dizygotic twins. * $p < .05$; ** $p < .01$; *** $p < .001$. [†] Spearman's rho was computed due to non-normal data. [‡] The 95% confidence interval bounds could not be estimated reliably. AE/CE = both AE and CE model fit the data equally well.*

Bivariate heritability of brain structure and components of prosocial behavior

First we investigated brain-behavior associations, as a starting point for our bivariate heritability analyses. We found a significant negative association between cortical thickness of precuneus and empathy (β = -.84, $t(396)$ = -3.15, p < .002). All other brain-behavior

associations were not significant. Additionally, we found a significant positive association between prosocial behavior and empathy (β = .44, t(482) = 6.96, p < .001). Both associations were significant at the Bonferroni-corrected p value ($\alpha_{corrected}$ = .003).

We then tested the contributions of genetics, shared environment and unique environment to covariance between structure of the social brain and prosocial behavior using bivariate ACE models. A complete overview of the path loadings (as visualized in Figure 3) can be found in Table 2. Overall, path loadings in the bivariate ACE models were comparable to those of the univariate ACE models.

To test for the shared variance between cortical thickness of precuneus and empathy, we found that a bivariate AE model was best fitting (see Table S3 for full model comparisons). A substantial part of the variance in cortical thickness of precuneus (path a_{11} = .58) and empathy (path a_{12} + path a_{22} = .71) was best explained by genetics. The remaining variance was best explained by unique environment/measurement error. Genetic effects explained 45% of the covariance between cortical thickness of precuneus and empathy (r_p = -.13), whereas 55% of the covariance was explained by unique environment/measurement error. Furthermore, we found that cortical thickness of precuneus and empathy were influenced by overlapping genetic (r_g = -.09) and unique environmental (r_e = -.21) factors to a minor extent, indicating that some factors account for both lower cortical thickness of precuneus and higher empathy (or vice versa).

For prosocial behavior and empathy, a bivariate AE model was best fitting (see Table S3 for full model comparisons). We found that a substantial part of the variance in prosocial behavior (path a_{11}=.45) and in empathy (path a_{12} + path a_{22} = .74) was best explained by genetics, with the remaining variance best explained by unique environment/measurement error. Genetic effects explained 47% of the covariance between prosocial behavior and empathy (r_p = .30), whereas 53% of the covariance was explained by unique environment/measurement error. Furthermore, we found that prosocial behavior and empathy were influenced by overlapping genetic (r_g = .25) and unique environmental (r_e = .42) factors, indicating that overlapping genetic and unique environmental factors account for some of the variance in prosocial behavior and empathy.

Table 2. Estimated contributions of genes (A), shared environment (C), and unique environment/measurement error (E) to covariance between structure of the social brain and prosocial behavior and empathy.

	$path_{11}$	$path_{12}$	$path_{22}$	r_p	r
Precuneus CT * Empathy					
A	0.58	0.01	0.7	0.45	-0.09
C	-	-	-	-	-
E	0.42	0.01	0.28	0.55	-0.21
Prosocial behavior * Empathy					
A	0.45	0.05	0.69	0.47	0.25
C	-	-	-	-	-
E	0.55	0.05	0.22	0.53	0.42

r_p = genetic/environmental contribution to covariance, r = genetic/environmental correlation. CT = cortical thickness.

Discussion

This study was driven by insights from prior studies showing protracted development of brain regions that are associated with prosocial behavior (mPFC, pSTS, TPJ and precuneus), but there is little understanding of what factors drive individual differences in the structure of theses brain regions. The first aim of the current study was therefore to investigate the contribution of genetics and shared environment on the social brain and components of prosocial behavior in 7-9-year-old children. Second, we tested whether there was shared genetic and environmentally driven covariance in the social brain and prosocial behavior. In our analyses of brain structure, we distinguished between surface area and cortical thickness, as these may be differentially sensitive to environmental influences (Noble et al., 2015; Piccolo et al., 2016). For surface area, we found influence of genetic factors for mPFC, pSTS and precuneus, whereas environmental influences were more pronounced for TPJ.

Additionally, we found a strong influence of genetics on cortical thickness of the precuneus, as well as influence of both genetics and environment on mPFC, TPJ and pSTS. On a behavioral level, we found that both prosocial behavior and empathy were strongly influenced by genetic factors. Finally, we found that covariance between cortical thickness of precuneus and empathy was partly explained by overlapping genetic factors. The discussion will first review the findings in social brain structure, followed by an interpretation of brain-behavior relations.

Genetic influences on structural properties of the social brain

Prior studies reported genetic influence on whole brain development in adults (Peper et al., 2007) and children (Peper et al., 2009; Teeuw et al., 2018), but this question was not yet addressed for regions in the social brain specifically, which have a prolonged developmental trajectory continuing until early adulthood for both cortical thickness and surface area (Mills et al., 2014). This led to the question whether the social brain was possibly more sensitive to influences from the environment (Blakemore & Mills, 2014).

Consistent with previous whole brain studies, there was pronounced evidence for genetic influences on brain structures in childhood, specifically for surface area, for all included regions in the social brain. Our estimates of genetic influence in mPFC, precuneus, pSTS and TPJ were comparable to prior studies that showed high estimates of genetic influence on overall surface area (71-92%; Ma et al., 2016; Panizzon et al., 2009; Winkler et al., 2010). In addition, studies investigating heritability of surface area of medial frontal regions also report similar estimates (ranging from 12-68%) in adolescents and adults (Ma et al., 2016; Panizzon et al., 2009; Winkler et al., 2010). We found that variances in surface area of mPFC, pSTS and precuneus were best explained by a combination of genetic factors and unique environment/measurement error.

In contrast to surface area, there was evidence for both genetic and environmental influences for cortical thickness of regions of the social brain in childhood, for all regions except for the precuneus. For the latter region, a combination of genes and unique environment/measurement error best explained variance in cortical thickness. This study complements previous studies that reported strong genetic influence on global cortical

thickness (52-81%), but more variable estimates for local cortical thickness (0-76%) across the lifespan (Lenroot et al., 2009; Panizzon et al., 2009; van Soelen et al., 2012; Winkler et al., 2010). Possibly, cortical thickness of the social brain, especially the mPFC, pSTS and TPJ, might be more susceptible to environmental influence compared to other brain regions (also see Blakemore and Mills (2014).

Interestingly, the TPJ in particular showed a pronounced influence of shared environment on both surface area and cortical thickness. The TPJ is consistently activated during social processing and social decision-making (Burnett, Bird, Moll, Frith, & Blakemore, 2008; van der Meulen et al., 2016; van Hoorn, Fuligni, Crone, & Galvan, 2016; van Hoorn, McCormick, Rogers, Ivory, & Telzer, 2018). Given that social processing is dependent on environmental input, the TPJ might therefore be particularly sensitive to the social environment. In addition, the involvement of the TPJ in social behavior changes over development (Güroğlu et al., 2009, 2014; Güroğlu et al., 2011; Tousignant et al., 2017; Will et al., 2015) and this region often shows brain-behavior correlations in functional neuroimaging research (Van Hoorn, Van Dijk, Guroglu, & Crone, 2016). Although structure of the TPJ follows similar developmental trajectories as other regions in the social brain (Mills et al., 2014) it is possible that differential genetic and environmental influences on structure of the TPJ become more pronounced over time, with environmental factors eventually having more impact on structure of the TPJ than genetic factors. Longitudinal twin-studies are necessary to investigate this hypothesis, as previous research has indicated a change in heritability with age (Lenroot et al., 2009).

An important question we could not address in the current study is whether surface area or cortical thickness is more strongly influenced by environmental factors. According to the radial unit hypothesis (Rakic, 1995) surface area and cortical thickness are driven by different developmental processes, possibly providing room for different contributions of genetic and environmental processes. In the current data set, there was no clear pattern showing that either cortical thickness or surface area were more strongly influenced by the environment, although there was slightly more evidence for shared environment influences on cortical thickness. However, our current sample was too small for draw concrete conclusions.

Genetic influences on brain-behavior associations

An important aim of this study was to relate the structural brain measures to prosocial behavior, as this behavior is often associated with the functioning of the social brain (Blakemore, 2008). For this purpose we focused on parent-report measures of prosocial behavior and empathy, as these measures encompass multiple contexts (Carlo & Randall, 2002) and reporting complex social behaviors such as prosocial behavior and empathy might be challenging for children (Richaud, Lemos, Mesurado, & Oros, 2017). We found that both prosocial behavior and empathy show strong influences of genetics, which is consistent with earlier studies reporting high estimates of heritability for parent-reported prosocial behavior (39-69%) and empathy (34-76%; Gregory et al., 2009; Knafo-Noam et al., 2015; Knafo & Plomin, 2006; Knafo et al., 2008; Melchers et al., 2016). Moreover, parent-reported prosocial behavior and empathy were positively associated, supporting previous findings of a multi-faceted perspective on prosocial behavior (Eisenberg, Hofer, Sulik, & Liew, 2014; Knafo-Noam et al., 2015). We found that not all of the covariance between prosocial behavior and empathy could be attributed to overlapping genetic and unique environmental factors, in line with findings by Knafo et al. (2008). This might indicate that prosocial behavior and empathy share a common origin, but that they are also driven by their unique biological and environmental processes.

We subsequently addressed the question whether there was covariance between structure of the social brain and components of prosocial behavior. We were primarily interested in brain regions that showed a consistent genetic factor, similar to what was observed for prosocial behavior and empathy. This was especially the case for the precuneus, for which we found strong influences of genetics on both surface area and cortical thickness. Indeed, cortical thickness of the precuneus was negatively associated with empathy. Findings from our bivariate analyses showed that decreased cortical thickness of the precuneus and increased empathy were, to a small extent, driven by overlapping genetic and unique environmental factors. Interestingly, previous studies have indicated a positive link between the precuneus and prosocial behavior and empathy, on both functional (Masten et al., 2010; Rameson, Morelli, & Lieberman, 2011; van der Meulen et al., 2018) and structural level (Thijssen et al., 2015). Since decreased cortical thickness indicates an increase in brain maturation (Mills et al., 2014; Wierenga et al., 2014), it is possible that the negative

association found in the current study indicates a link between a matured precuneus and increased prosocial behavior in middle childhood. Prior studies have pinpointed the precuneus as an important region for evaluating both the self and other persons (Ochsner et al., 2005; Pfeifer et al., 2007). Possibly, the precuneus plays a crucial role in differentiating between self and other, thereby facilitating perspective taking in a social situation. In addition, the precuneus is involved in autobiographical memory (for review see Cavanna and Trimble (2006), which might enable an accurate recall of one's capability to help another in distress. The involvement of the precuneus in both perspective taking and recall of one's own capabilities might make the precuneus an essential brain region for prosocial behavior in childhood. Our finding in this specific age range is particularly important to better understand the starting point of the large-scale brain development of adolescence (Mills et al., 2016; Vijayakumar et al., 2016; Wierenga et al., 2014).

Limitations

The current study had several limitations that should be addressed in future research. First, although we differentiated between prosocial behavior and empathy we did not further account for various subtypes of prosocial behavior. Within the area of prosocial behavior, researchers distinguish between context-specific costly prosocial behavior (helping or sharing at the cost of one-self) and non-costly prosocial behavior (helping and sharing to benefit others but at no cost for self; Fehr et al., 2008), and general prosocial behavior (the intention to help, comfort, or share with others). For the current study, we have chosen to investigate prosocial behavior across contexts, rather than a specific situation, thereby providing a more general perspective on prosocial behavior but more limited in terms of potential response biases of the informants. Furthermore, although mentalizing and perspective taking are commonly associated with the social brain and prosocial behavior, we did not include behavioral measures of perspective taking in our design. Future research should aim to disentangle genetic and environmental effects for various types of prosocial behavior, in order to achieve a more comprehensive understanding of this multidimensional construct.

Second, we limited our selection of regions of interest in the social brain to four key regions (mPFC, TPJ, pSTS, and precuneus). Although this ROI driven approach increases statistical power, for a more comprehensive understanding of genetic and environmental influences on the social brain it might be interesting to also include regions such as the anterior cingulate cortex (ACC), amygdala, and anterior insula in future studies, as these regions are also involved in social cognition and behavior (Blakemore, 2008). Third, the current study is cross-sectional and therefore no interpretations can be made regarding the relationship between structure of the social brain and prosocial behavior across development. In order to better understand influences of genetics and environment on brain development and brain-behavior associations over time a longitudinal design is required (Brans et al., 2010; Mills et al., 2014; Shaw et al., 2006). Finally, although the current sample size was sufficient to investigate surface area and cortical thickness of the social brain, it might have been underpowered in fitting the bivariate ACE models. Our initial findings should therefore be taken as a starting point for future research.

Conclusion

The current study contributes to the current theoretical framework by investigating the influence of genetics and environment on brain regions that are of particular interest for (pro)social behavior. Moreover, brain-behavior relationships were studied in a relatively young sample, around or prior to gray matter changes in adolescence. This twin-study confirmed the hypothesis that regions of the social brain showed distinguishable influences of genetics, shared environment, and unique environment, with more influence of shared environment on cortical thickness than on surface area. In addition, we found that especially the TPJ might be more susceptible to environmental and social influences. Structural properties of the precuneus showed strong influence of genetics, which partly overlapped with genetic influence on parent-reported empathy, indicating that similar biological and environmental processes drive variance in this brain-behavior relationship. An important question for future research is whether behavioral interventions aimed at increasing prosocial behavior have an impact on the developmental trajectory of the social brain

regions, which would provide stronger evidence for an impact of environment on brain and behavioral development.

Supplementary Materials

Table S1. Questionnaire structure after principal component analysis.

Question	Original questionnaire
Prosocial subscale	
Considerate of other people's feelings	SDQ
Shares readily with other children	SDQ
Helpful if someone is hurt	SDQ
Kind to younger children	SDQ
Often volunteers to help others	SDQ
Will try to comfort or reassure another in distress	MC
Likely to offer toys or candy to a crying playmate even without parental suggestion	MC
Likely to show spontaneous nurturing and care-giving behavior toward an animal	MC
Can tell at just a glance how others are feeling	MC
Likely to ask, "What's wrong?" when seeing someone in distress	MC
Will feel sorry for other people who are hurt, sick, or unhappy	MC
Empathy subscale	
Acts upset when he/she sees a hurt animal	MC
Rarely cries or looks upset when watching a sad TV show (recoded)	MC
Gets angry at aggressor, "Bad Guy", who hurts a TV character	MC
Is upset by stories in which characters are hurt or die	MC
Is not likely to become upset if a playmate cries (recoded)	MC
Not included in new subscales	
May occasionally tease a pet if unsupervised (recoded)	MC
Feels good when good things happen to movie characters	MC

Note. SDQ = Strenghts and Difficulties Questionnaire; MC = My Child Questionnaire

Table S2. Comparison of univariate ACE models for structural properties of regions of the social brain and components of prosocial behavior.

Outcome variable	model	A^2	C^2	E^2	LRT	AIC
Surface area						
mPFC	ACE	0.5	0	0.5		3190.10
	AE*	**0.5**	**-**	**0.5**	**< .001**	**3188.10**
	CE	-	0.34	0.66	7.25	3195.35
	E	-	-	1	> 21.72	3215.06
TPJ	ACE	0.24	0.23	0.53		-1674.82
	AE*	**0.5**	**-**	**0.5**	**1.42**	**-1675.40**
	CE*	**-**	**0.41**	**0.59**	**1.06**	**-1675.76**
	E	-	-	1	> 32.46	-1644.94
	ACE	0.51	0.14	0.73		-1778.69
pSTS	**AE***	**0.65**	**-**	**0.35**	**0.43**	**-1780.26**
	CE	-	0.52	0.48	7.15	-1773.54
	E	-	-	1	> 56.87	-1718.66
	ACE	0.81	0	0.19		-1964.11
Precuneus	**AE***	**0.81**	**-**	**0.19**	**< .001**	**-1966.11**
	CE	-	0.6	0.4	36.01	-1930.10
	E	-	-	1	> 80.71	-1851.40
Cortical thickness						
mPFC	ACE	0.16	0.06	0.78		-540.43
	AE*	**0.23**	**-**	**0.77**	**0.07**	**-542.36**
	CE*	**-**	**0.18**	**0.82**	**0.3**	**-542.13**
	E	-	-	1	> 5.80	-538.32

Table S2. Continued.

Outcome variable	model	A^2	C^2	E^2	LRT	AIC
TPJ	ACE	0.08	0.22	0.68		-894.38
	AE*	**0.34**	**-**	**0.66**	**1.05**	**-895.33**
	CE*	**-**	**0.28**	**0.72**	**0.09**	**-896.28**
	E	-	-	1	> 13.88	-883.45
pSTS	ACE	0.27	0.02	0.72		-1004.56
	AE*	**0.29**	**-**	**0.71**	**0.004**	**-1006.56**
	CE*	**-**	**0.24**	**0.76**	**0.81**	**-1005.76**
	E	-	-	1	> 10.91	-996.85
Precuneus	ACE	0.55	0	0.45		-1266.28
	AE*	**0.55**	**-**	**0.45**	**< .001**	**-1268.28**
	CE	-	0.35	0.65	10.86	-1257.43
	E	-	-	1	> 24.18	-1235.25
Parent reported behavior						
Prosocial	ACE	0.45	0	0.55		-329.76
behavior	**AE***	**0.45**	**-**	**0.55**	**< .001**	**-331.76**
	CE	-	0.27	0.73	11.89	-319.86
	E	-	-	1	> 18.69	-303.18
Empathy	ACE	0.62	0.12	0.26		-61.06
	AE*	**0.74**	**-**	**0.26**	**0.51**	**-62.55**
	CE	-	0.61	0.39	19.34	-43.72
	E	-	-	1	> 113.60	67.88

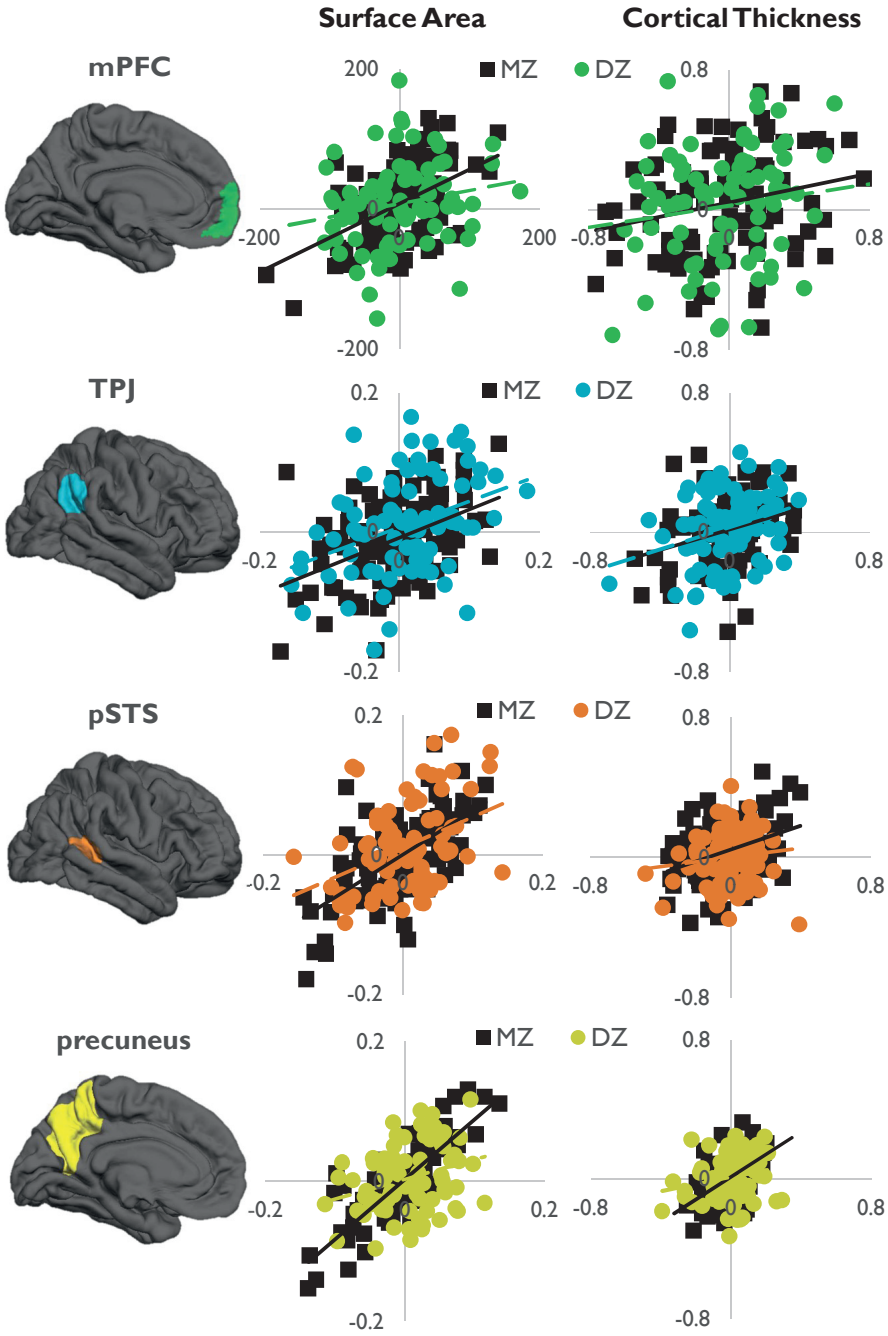
Best fitting models are indicated by an asterisk (*) and bold font.

Table S3. Comparison of bivariate ACE models for structural properties of the precuneus and components of prosocial behavior.

Model	-2LL	df	AIC	LRT	*p*
*Precuneus CT * Empathy*					
Saturated Cholesky	47.4	656	-1264.6		
ACE	68.57	673	-1277.43	21.17	0.22
AE*	**68.74**	**676**	**-1283.26**	**0.17**	**0.98**
CE	93.62	676	-1258.38	25.05	< .001
E	191.98	679	-1166.02	123.41	< .001
*Prosocial behavior * Empathy*					
Saturated Cholesky	1461.93	944	-426.07		
ACE	1475.72	961	-446.28	13.79	0.68
AE*	**1478.17**	**964**	**-449.83**	**2.45**	**0.48**
CE	1504.25	964	-423.75	28.53	< .001
E	1654.31	967	-279.69	178.58	< .001

Best fitting models are indicated by an asterisk (*) and bold font.

Figure S1 (on right page). Visualisation of within-twin correlations for surface area and cortical thickness of mPFC, TPJ, pSTS and precuneus. MZ = monozygotic; DZ = dizygotic.


Surface Area
Cortical Thickness
mPFC
TPJ
pSTS
precuneus
MZ
DZ
200
-200
0.2
-0.2
0.8
-0.8