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

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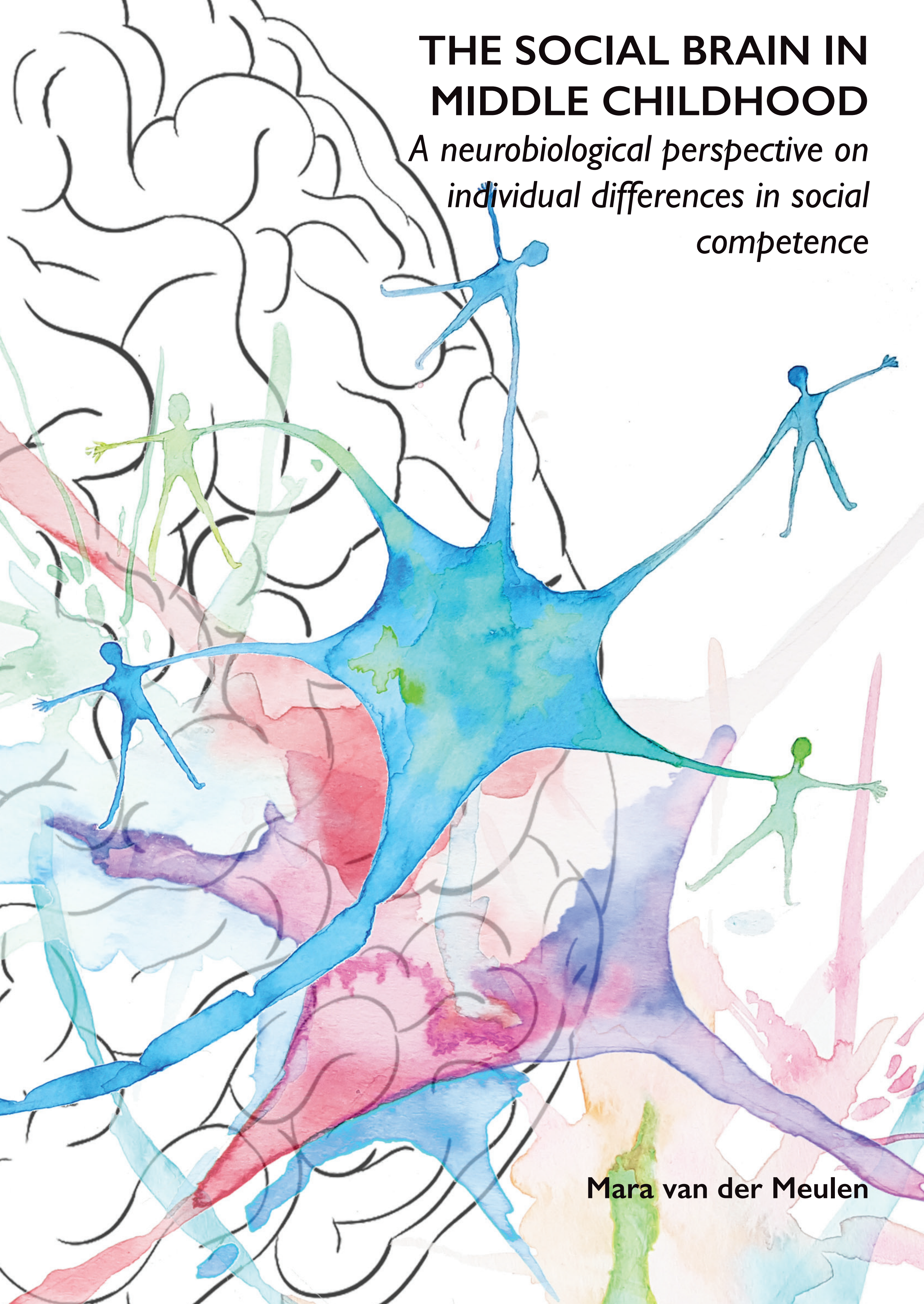

Mara van der Meulen

THE SOCIAL BRAIN IN MIDDLE CHILDHOOD

A neurobiological perspective on individual differences in social competence

Mara van der Meulen

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THE SOCIAL BRAIN IN MIDDLE CHILDHOOD

A neurobiological perspective on individual differences in social competence

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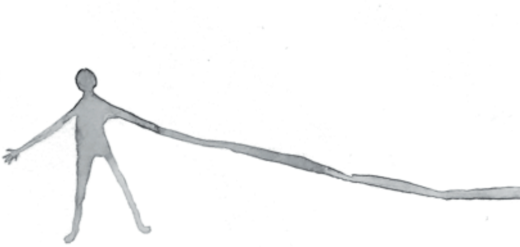
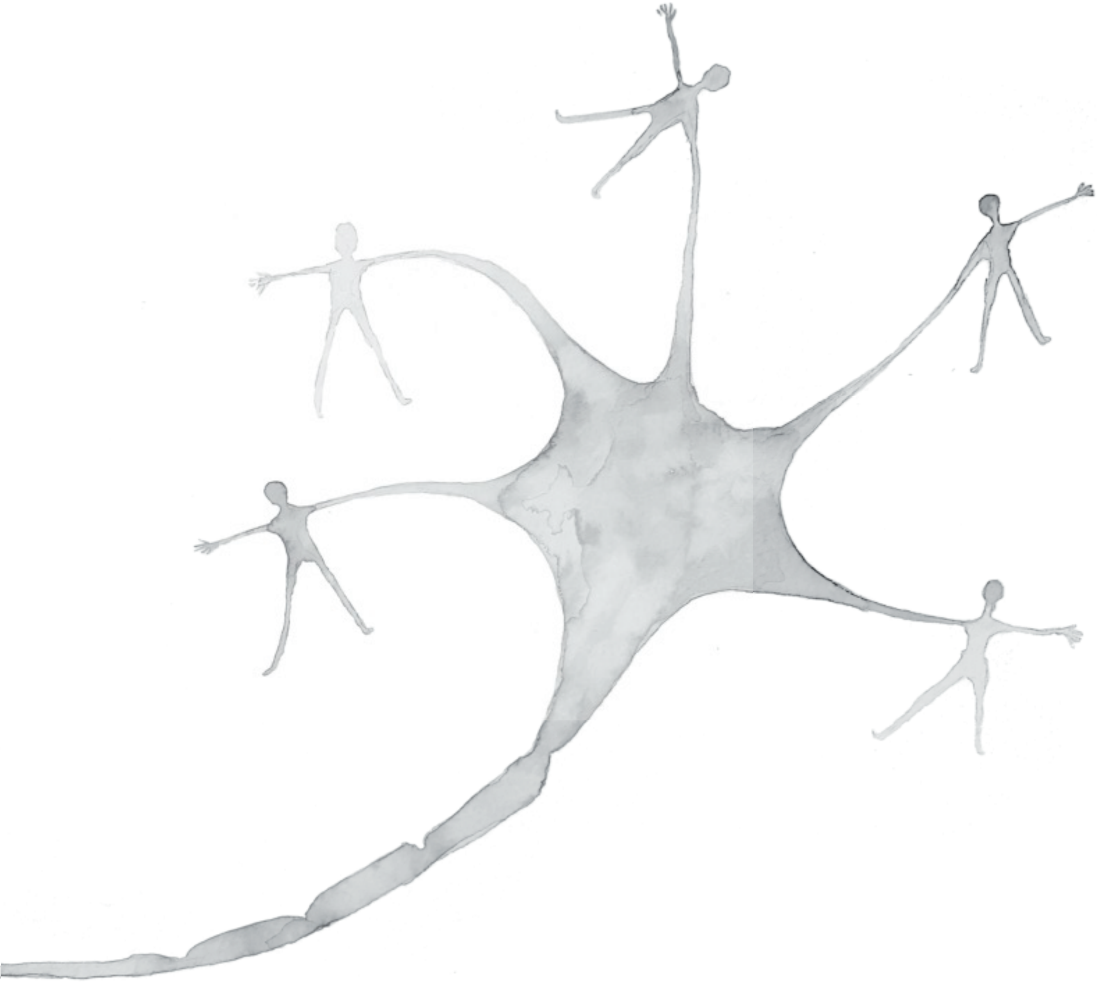


CHAPTER 1

General introduction



Introduction

A fundamental part of human development is the social relationship formed with other individuals. Numerous studies have indicated that people with a strong social network usually live longer, feel happier and are healthier than those who feel lonely (Goswami, 2012; Holt-Lunstad, Smith, & Layton, 2010; Reblin & Uchino, 2008; Smith & Christakis, 2008). In order to establish such a social network it is of key importance for children to develop social competence, or the ability to fulfill both others' and own needs in a social context (Rubin & Rose-Krasnor, 1992). Children can fulfill others' and their own needs by showing positive behavior such as helping another in distress (Eisenberg, Fabes, & Spinrad, 2006). However, if children are excluded from social interaction from an early age onwards, their own social needs are neglected and they might fail to develop the social competence that is necessary for social functioning later in life (Rubin, Bukowski, & Parker, 2006).

The development of social competence is possible due to the specific wiring of the human brain, which has adapted to facilitate the need for social interaction (Dunbar & Shultz, 2007). It is thought that social competence, as well as the underlying neural processes, are a result of a constantly changing interplay between genes and environment (Ebstein, Israel, Chew, Zhong, & Knafo, 2010). Middle childhood (age 6-12) in particular is seen as a key period in the development of social competence (Del Giudice, Angeleri, & Manera, 2009). Therefore, it is important to better understand why some children in this developmental stage thrive socially, and how these individual differences are related to underlying neural processes.

The goal of this thesis is thus to examine the processes involved in social competence in middle childhood, using a combination of experimental paradigms and parent-report questionnaires. In addition to behavioral measures, I have used neuroimaging techniques in order to better understand underlying processes in social competence. Furthermore, I have used a behavioral genetics approach to unravel influences of genetics and environment on social competence and associated neural processes. In this first chapter, I start with an introduction of middle childhood as a key period in human social development. This is followed by a section on factors contributing to social competence, and a section on the brain regions that support social competence. Finally, I will highlight the additional value of heritability research in the understanding of social competence. The final section of this introduction describes how the individual chapters of this thesis contribute to a neurobiological perspective on social competence in middle childhood.

Middle childhood

Traditionally, middle childhood (age 6-12 years) has been seen as a period of relative tranquility in development. Middle childhood is preceded by early childhood (age 0-6 years), the developmental stage for obtaining basic skills including motor control, speech and initial academic performance, such as reading, writing, and simple mathematics (Siegler, Deloache, & Eisenberg, 2006). Directly after middle childhood follows adolescence (age 12-18 years), the period in life characterized by changes in individual exploration and identity development (Crone & Dahl, 2012), and increased independence from parents (Nelson, Jarcho, & Guyer, 2016).

Middle childhood provides the groundwork for the large-scale developments of adolescence and therefore forms a unique and crucial developmental stage. During middle childhood children still receive attention and care from their caregivers, but they also start to develop themselves as individual members of society by discovering their interests, likes and talents (Del Giudice, 2014; Erikson, 1994). In addition, children show a large increase in complex cognitive skills such as self-regulation (Del Giudice, 2014; Steinbeis, Singer, Fehr, & Haushofer, 2014), as well as an rising awareness of their own feelings and mental states (Mah & Ford-Jones, 2012). These personal developments go hand in hand with social developments such as an increased understanding of social norms and values, such as notions of fairness and morality (Jambon & Smetana, 2014; Piaget, 1932). Although younger children are aware of the existing social norms and values, it is not until middle childhood that these norms are internalized and enforced in the communication with others (McAuliffe, Blake, Steinbeis, & Warneken, 2017; Smith, Blake, & Harris, 2013). The combination of increased personal and social skills allows for an increase in social competence, along with a shift in peer interaction from merely playing together with peers to the formation of dyadic friendships that are often based on shared interests (Del Giudice et al., 2009). The social status derived from those friendships often carries over well into adulthood, thereby highlighting the importance of developing social competence at an early age.

Other- and self-oriented factors in social competence

Social competence

Following the definition by Rubin and Rose-Krasnor (1992) social competence should be seen as a two-pronged ability: a socially competent individual is able to understand both another individual's interests and needs, as well as their own interests and needs. From infancy onwards children are sensitive to each other's emotions (Decety & Jackson, 2004; Hoffman, 1987). However, additional affective and socio-cognitive skills are needed to actually understand another child's distress and act accordingly. Affective skills include emotion understanding (the ability to correctly label an emotion; Reschke, Walle, & Dukes, 2017) and empathy (the ability to feel the emotion another child is feeling; Eisenberg, Spinrad, & Knafo-Noam, 2015), whereas socio-cognitive skills include perspective taking (the ability to understand that another child's perception might differ from one's own perception; Penner & Finkelstein, 1998) and self-regulation (Steinbeis, 2018).

Prosocial behavior

Prosocial behavior is seen as a component of social competence that provides a foundation for positive and reciprocal relationships (Over, 2016; Steinbeis, Bernhardt, & Singer, 2012). It can be broadly defined as a voluntary act to help another individual (Eisenberg et al., 2006; El Mallah, 2019; Padilla-Walker & Carlo, 2014), such as helping someone in need or distress, sharing resources, cooperating or comforting. In addition, those who show more prosocial behavior also show more academic achievement (Caprara, Barbaranelli, Pastorelli, Bandura, & Zimbardo, 2000; Wentzel, 1993), less externalizing behavior (Padilla-Walker, Carlo, & Nielson, 2015) and a better sense of well-being (Eisenberg et al., 2015) thereby extending the benefits of prosocial behavior past the social environment. Early signs of prosocial behavior are already apparent when children are 18 months old (Warneken & Tomasello, 2006), but prosocial behavior continues to develop throughout childhood along with the development of affective and cognitive abilities (Steinbeis & Over, 2017). Displays of prosocial behavior vary across contexts (Carlo & Randall, 2002; Dunfield, Kuhlmeier, O'Connell, & Kelley, 2011), as one might be generous with donating money to charity, but be more hesitant in comforting a stranger. Moreover, the motivations underlying prosocial behavior are thought to vary from altruistic (with no regard for own benefit) to self-serving intentions (Eisenberg & Spinrad, 2014). In line with this diversity in behavior, it should be noted that the best practice for measuring prosocial behavior is sometimes debated and differs between researchers (see El Mallah (2019) for review). For example, some researchers prefer to focus on observable prosocial behavior (using observational or experimental measures; Fehr, Bernhard, & Rockenbach, 2008; Güroğlu, van den Bos, & Crone, 2014), whereas other researchers are more interested in the tendencies underlying prosocial behavior, regardless of the outcome being more beneficial for the actor or the recipient (using questionnaires; Knafo-Noam, Uzefovsky, Israel, Davidov, & Zahn-Waxler, 2015). In this thesis I have combined two measurement types in order to measure various aspects of prosocial behavior. In **chapters 2, 3** and **4** I used an experimental task to measure prosocial behavior in a specific situation of observed social exclusion, and in **chapter 5** I have used a parent-reported measure of prosocial behavior to investigate prosocial behavior towards different recipients and across contexts.

Social exclusion

In contrast to children who can increase their social competence in a supportive peer environment, children who already have a lower level of social competence might be excluded from the peer group (Rubin et al., 2006). Being socially excluded results in internalizing and externalizing problems (Ladd, 2006), and feelings of loneliness (Boivin, Hymel, & Bukowski, 1995; Cassidy & Asher, 1992). In the long term, social exclusion is linked to feelings of depression (Ladd & Troop-Gordon, 2003), decreased academic performance (DeRosier & Lloyd, 2010; Véronneau, Vitaro, Brendgen, Dishion, & Tremblay, 2010) and decreased overall well-being, again extending the results of peer group interaction far past the social environment. Often children who are rejected by their peers either internalize their feelings and hide their emotions, or they lash out, thus behaving against social norms and giving peers cause for further rejection (Ladd, 2006; Rubin et al., 2006).

This in turn leaves a rejected child with less possibilities to obtain the social competence that they so desperately need to connect better with their classmates. It might therefore not be surprising that children who were excluded in childhood are also more likely to be socially excluded later in life (Hardy, Bukowski, & Sippola, 2002).

Across the lifespan, being socially excluded is such a salient event that even a simple virtual ball-tossing game is enough to elicit feelings of exclusion. The Cyberball Game, designed by Williams, Cheung, and Choi (2000) has often been used to create a virtual situation of social exclusion, in order to better understand the psychological consequences of exclusion (Masten et al., 2009; Will, van Lier, Crone, & Güroğlu, 2016). In **chapters 3** and **4** I have used an adapted version of the Cyberball Game (based on Riem, Bakermans-Kranenburg, Huffmeijer, and van IJzendoorn (2013)) to examine responses to short-term social exclusion from a group of virtual players.

Neural networks supporting social competence

In the last decades structural and functional magnetic resonance imaging (MRI) has been employed to further investigate underlying neural processes in social competence. Structural neuroimaging studies have shown that both grey matter (neurons) and white matter (connections between neurons) continue to increase from infancy throughout childhood. In adolescence, a large number of neurons are eliminated following the "use it or lose it" principle: important connections in the brain are kept to be refined and optimized, whereas redundant connections are discarded (Gogtay et al., 2004; Wierenga, Langen, Oranje, & Durston, 2014). This means that even though the brain has already reached its maximum volume at the start of middle childhood (Del Giudice, 2014), neural development continues until adulthood. In the investigation of social behavior, functional neuroimaging studies have consistently shown that a network of specific brain regions (known as the social brain) has developed to support the complex functions needed for human interaction (Blakemore, 2008; Frith & Frith, 2003). The social brain includes regions that are involved in both the socio-cognitive and affective processes of social competence (also see Figure 1).

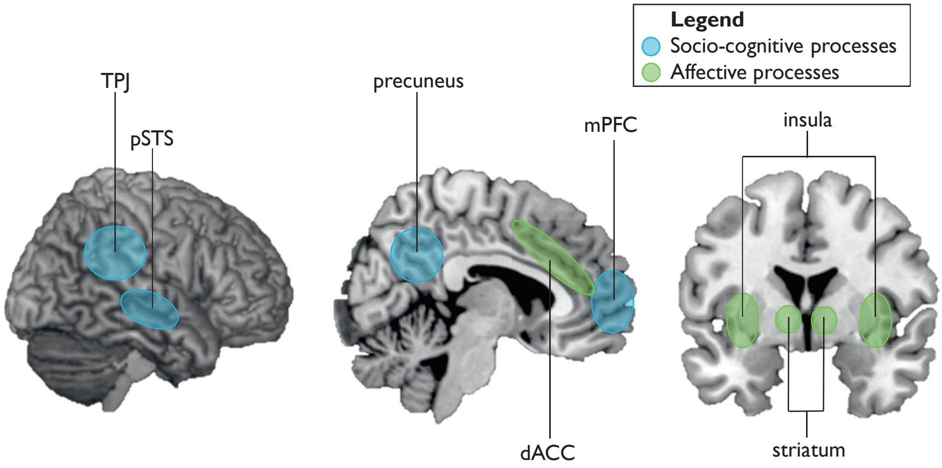


Figure 1. *Visualization of the social brain. Areas highlighted in blue are involved in socio-cognitive processes; temporo-parietal junction (TPJ), posterior superior temporal sulcus (pSTS), precuneus, and medial prefontal cortex (mPFC). Areas highlighted in green are involved in affective processes; dorsal anterior cingulate cortex (dACC), insula, and striatum. Figure adapted from Will and Güroğlu (2016).*

The socio-cognitive processes of social competence, including the ability to take someone else's perspective, are supported by regions such as the medial prefrontal cortex (mPFC), temporo-parietal junction (TPJ), posterior superior temporal sulcus (pSTS) and the precuneus (Blakemore, 2008). Specifically, the TPJ is thought to play a role in perspective taking (Carter & Huettel, 2013; Will, Crone, & Guroglu, 2015), whereas the pSTS is involved in the processing of general social information such as biological motion and speech (Frith & Frith, 2007; Frith & Frith, 2003; Van Overwalle & Baetens, 2009). The mPFC might be more involved in thinking about one's personal and others' mental states (Frith & Frith, 2007; Frith & Frith, 2003; Van Overwalle & Baetens, 2009). The role of the precuneus in social competence is less clear, as this region is both implicated in the retrieval of social information (Pfeifer, Lieberman, & Dapretto, 2007) as well as in empathic responding (Masten, Morelli, & Eisenberger, 2011). Together, these regions have been consistently shown to support prosocial behavior (Güroğlu, van den Bos, & Crone, 2009; Güroğlu, van den Bos, van Dijk, Rombouts, & Crone, 2011; Tousignant, Eugène, Sirois, & Jackson, 2017; Will et al., 2015).

In addition to socio-cognitive processes, the affective processes of social competence encompass processing of emotions. These affective processes are supported by regions such as the anterior insula (AI), dorsal anterior cingulate cortex (dACC), and the ventral striatum (VS). The anterior insula and dACC are particularly important for detecting salient events (Menon & Uddin, 2010; Seeley et al., 2007), and have been implicated in the processing of both positive and negative social events (Davey, Allen, Harrison, Dwyer, & Yucel, 2010; Eisenberger, Lieberman, & Williams, 2003). The ventral striatum on the other hand is seen as a reward center in the brain (Delgado, 2007; Lieberman & Eisenberger, 2009) and might therefore be more involved in positive social experiences such as voluntary sharing goods (Harbaugh, Mayr, & Burghart, 2007). Neuroimaging research has consistently shown that the experience of social exclusion is associated with increased neural activity in the AI and dACC (Cacioppo et al., 2013; Eisenberger et al., 2003; Masten et al., 2009; Rotge et al., 2015). Additionally, self-reported levels of distress have been shown to positively correlate to neural activity in AI and dACC, thereby further establishing the association between the negative experience of social exclusion and concurrent neural activity (Masten et al., 2009). Interestingly, observing social exclusion of someone else is associated with the same neural network as experiencing social exclusion (Masten, Eisenberger, Pfeifer, & Dapretto, 2010; Meyer et al., 2013)

In an investigation of brain structure it has been shown that regions in the social brain continue to develop well into adolescence (Mills, Lalonde, Clasen, Giedd, & Blakemore, 2014). This finding is accompanied by functional MRI studies showing that brain activity for social events is different across development. For example, the TPJ shows increasingly stronger activation during prosocial behavior over the course of development (Güroğlu et al., 2009, 2014; Güroğlu et al., 2011; Tousignant et al., 2017; Will et al., 2015). It is important to note that the relationship between brain and behavior is bidirectional: brain structure and function can be affected by social factors such as a lack of positive parenting (Belsky & de Haan, 2011) or a lack of positive peer experiences (Will et al., 2016), but in turn alterations in brain structure can also affect a child's social behavior (Beauchamp, Dooley, & Anderson, 2013). Given the importance of middle childhood for the development of social competence, it is credible that individual differences in social behavior in this particular developmental stage are reflected in function and structure of the social brain. In this thesis, I examined the association between prosocial behavior and brain function, as well as between social exclusion and brain function, in **chapters 2, 3** and **4** (studying both adult and middle childhood samples)**.** In the same middle childhood sample, I studied the association between prosocial behavior and brain structure in **chapter 5.**

Heritability of social brain and behavior

A method to investigate whether middle childhood is a particularly sensitive period in terms of social environmental influences is by assessing the heritability of social competence in this particular age range. That is, to assess whether variance in social competence can be best explained by genetic factors, environmental factors, or a combination of the two. In short, genetic factors usually consist of variations in single or multiple genes, whereas environmental factors can be divided into shared (e.g. parenting or home environment) or unique (i.e. specific for an individual child) factors (McLoughlin, Ronald, Kuntsi, Asherson, & Plomin, 2007; Plomin, DeFries, McClearn, & McGuffin, 2001).

Estimations of genetic and environmental influence can be made in twin samples, as those samples allow for a comparison of individuals with 100% shared genes (i.e. monozygotic (MZ) twins) and individuals with 50% shared genes (i.e. dizygotic (DZ) twins). In those comparisons, it is assumed that both MZ and DZ twins are raised together and therefore share the home environment to a similar extent. The finding of a high positive association between MZ twins than between DZ twins is an indication of a strong genetic influence. If MZ twins and DZ twins show equally high associations then the behavior is probably driven by shared environmental factors, due to the shared home environment for both MZ and DZ twins. If there is no association between either MZ or DZ twins, then the variance in behavior is probably driven by unique environmental factors or by measurement errors (Knafo-Noam, Vertsberger, & Israel, 2018).

Using this method, studies have shown that half of the variance across the whole range of human behavior can be explained by genetics (Polderman et al., 2015). More specifically for factors underlying social competence, it has been shown that up to half of the variance in prosocial behavior and empathy could be attributed to genetic factors, with the remainder of the variance best explained by shared and unique environmental factors (Gregory, Light-Hausermann, Rijsdijk, & Eley, 2009; Knafo-Noam et al., 2015; Knafo et al., 2009; Melchers, Montag, Reuter, Spinath, & Hahn, 2016). Corresponding to heritability estimates of behavior, it has been found that different measures of brain structure are also strongly driven by genetic factors (Ma et al., 2016; Panizzon et al., 2009; Peper, Brouwer, Boomsma, Kahn, & Hulshoff Pol, 2007; Teeuw et al., 2018), although heritability estimates of brain functioning in social contexts have not been investigated in detail.

It is important to note that the relative influence of genes and environment on behavior and brain is not constant throughout development (Davis, Haworth, & Plomin, 2009; Lenroot & Giedd, 2008). For example, research has indicated that for both prosocial behavior and brain structure genetic factors become increasingly more influential as children grow older (Knafo & Plomin, 2006; Lenroot & Giedd, 2008). Moreover, heritability estimates differ for different components in prosocial behavior (Knafo-Noam et al., 2015). In this thesis I studied heritability of prosocial behavior and social exclusion in an experimental paradigm, along with associated brain function, in **chapter 4**. In **chapter 5** I investigated heritability of parent-reported prosocial behavior and its association with brain structure.

Outline of this thesis

In this thesis I report the results from four empirical studies that I have conducted to investigate individual differences in reactions to the social environment, using an experimental paradigm, parent-report questionnaires, and functional and structural neuroimaging. A graphical outline of the concepts and measurements discussed in the four subsequent chapters is shown in Figure 2.

In **chapter 2** the results of an fMRI version of a four-player Prosocial Cyberball Game (PCG) in adults are presented. Using an experimentally induced situation of observed exclusion, I aimed to disentangle neural responses during prosocial behavior from neural responses to observed social exclusion. This adult study was used as a starting point for the next chapters. In **chapter 3** the PCG was validated as a fMRI paradigm in middle childhood. Here I examined behavioral and neural responses to observed social exclusion, as well as neural responses to experienced social exclusion. I employed a test-replication design and meta-analytic approach to investigate behavioral and neural responses across three samples (all 7-10 years old). In **chapter 4** I combined neuroimaging and behavioral genetics in a large middle childhood sample, to investigate contributions of genes and environment on the behavioral and neural responses to the PCG. In **chapter 5** I extend the investigation of heritability of prosocial behavior and neural correlates in the same middle childhood sample, by describing unique and shared influences of genes and environment on parent-reported prosocial behavior and structural measures of social brain regions (i.e. mPFC, TPJ, pSTS, precuneus). Finally, in **chapter 6** I summarize and discuss the findings of the empirical chapters and provide an overview of the implications of these findings.

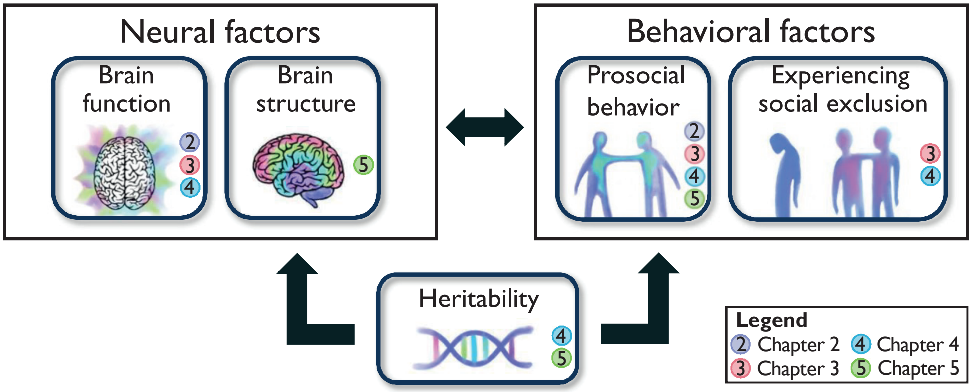


Figure 2. *Visualization of the relationship between behavioral, neural and heritability factors in social competence presented in the current thesis.*

CHAPTER 2

Neural correlates of prosocial behavior: Compensating social exclusion in a four-player Cyberball Game

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Abstract

Prior studies demonstrated contributions of the insula and dorsal anterior cingulate cortex (dACC) for both experiencing and observing social exclusion, but it is not yet well understood how the brain processes the compensation of exclusion, as is observed in prosocial helping. Here, we tested if social brain regions, specifically the medial prefrontal cortex (mPFC) and temporal parietal junction (TPJ) are involved when individuals show prosocial behavior towards excluded others. For this purpose, 23 female participants played a four-player Cyberball Game in which participants could toss balls to each other. During the exclusion game, two players excluded one of the other players. When participants observed exclusion by others, they showed elevated activity in the insula, consistent with prior studies. However, when they tossed the ball to the excluded player, they showed increased activation in the TPJ, consistent with the hypothesis that prosocial behavior is associated with social reasoning. In addition, tossing to the excluded player was associated with increased activity in the nucleus accumbens (NAcc). Given that prior studies reported that the NAcc is involved in experiencing rewards, this may suggest a warm glow for showing prosocial compensation behavior when helping excluded others.

Introduction

Prosocial behavior involves helping, sharing or comforting others without personal benefit, and is an important component of social life. Prior studies showed that humans compensate for others who are in need, for example by sharing distress of observed exclusion (Masten et al., 2010; Masten et al., 2011; Will, van den Bos, Crone, & Guroglu, 2013) and by helping victims of exclusion (Riem et al., 2013). Prior studies also documented that seeing someone in distress causes feeling of personal distress as well (Wesselmann, Bagg, & Williams, 2009). Knowing you can do something to alleviate another person's distress can lead to acts of prosocial behavior, as already seen in very young children (Roth-Hanania, Davidoy, & Zahn-Waxler, 2011). Yet, prior studies have not attempted to separate the different components of sharing social pain and compensating others, possibly because it is difficult to disentangle these effects using behavioral measures. Neuroimaging may prove a powerful tool to dissociate which brain regions are sensitive to separate phases of observing social pain of others and actively compensating social exclusion.

Several earlier neuroimaging studies have shown the link between observed distress and experienced personal distress. These studies typically show that observing others in social pain activates similar brain regions as when being excluded (Masten, Eisenberger, Pfeifer, Colich, & Dapretto, 2013; Masten et al., 2010; Meyer et al., 2013; Novembre, Zanon, & Silani, 2015). Prior studies have made use of a virtual ball tossing game referred to as Cyberball, in which three players participate in a computerized game of ball tossing. During the first round all players participate and toss the ball to each other. In subsequent rounds the computer controlled game ensures that one player no longer receives the ball, thereby creating a situation of social exclusion (Williams et al., 2000). When individuals are excluded by others in Cyberball, they show increased neural activity in the dorsal anterior cingulate cortex (dACC) and bilateral insula (Eisenberger et al., 2003; Masten et al., 2009). Furthermore, there is consistent evidence for the role of dACC and bilateral insula in experiencing social rejection (for meta-analyses see Cacioppo et al. (2013); Rotge et al. (2015)). The meta-analyses also revealed other areas implicated in social rejection, such as the left orbitofrontal cortex. However, the orbitofrontal cortex plays a more general role in human decision making and signaling value and might therefore be considered less specific to the experience of social rejection (Stalnaker, Cooch, & Schoenbaum, 2015). The observation of social exclusion has also been associated with activity in the bilateral insula and dACC, although more so when a friend (compared to a stranger) is being excluded (Meyer et al., 2013). Interestingly, several studies have reported that activity in the insula is correlated with subsequent prosocial behavior (Masten et al., 2010; Masten et al., 2011) and that activity in both bilateral insula and dACC is associated with empathy traits (Masten et al., 2011; Meyer et al., 2013).

A separate set of neuroimaging studies focused on the role of helping, or compensating for social pain of others. When participants are given the opportunity to interact with the players from a previously observed Cyberball game, participants tend to show more prosocial behavior towards the excluded player than towards the excluding players (Masten et al., 2010; Masten et al., 2011; Will et al., 2013). Prior studies tested how participants allocate points to others after playing Cyberball and being excluded. It was found that considering how to react towards excluders was associated with more activity in the temporal-parietal junction (TPJ; Moor et al., 2012; Will et al., 2015)), a region associated with perspective taking (Carter & Huettel, 2013). The TPJ is often interpreted as a brain region which is part of a social brain network, including also the medial PFC and superior temporal sulcus (STS), and which is activated when individuals think about intentions of others (Blakemore, 2008; Burnett & Blakemore, 2009). In addition, it was previously found that sharing with others results in increased activity in the ventral striatum/nucleus accumbens (NAcc), which is thought to be a reward center of the brain (Delgado, 2007; Guroglu, Will, & Crone, 2014; Lieberman & Eisenberger, 2009; Telzer, 2016). Possibly, prosocial behavior elicits a rewarding feeling, or a warm glow (Harbaugh et al., 2007).

Taken together, prior studies showed that the insula and dACC are active when experiencing exclusion or observing exclusion of others (Eisenberger et al., 2003; Meyer et al., 2013) and medial PFC, TPJ and NAcc are activated when compensating others or considering the reactions towards excluders (Lieberman & Eisenberger, 2009; Moor et al., 2012; Will et al., 2015), but no study to date directly compared the neural correlates of observing exclusion and helping in a prosocial game. The goal of this study was to test the relative role of these regions in a prosocial Cyberball game in which participants can help excluded players. During this game, the participants observe another player being excluded, while having the opportunity to compensate for the excluding behavior by tossing the ball to the excluded player.

First, we predicted that participants would take the opportunity to compensate for observed exclusion by acting prosocially towards an excluded person (Masten et al., 2010; Masten et al., 2011; Riem et al., 2013; Will et al., 2013). We expected that this behavior would be related to self-reported empathy (Masten et al., 2011). Second, we predicted that the observation of exclusion would lead to increased activity in the bilateral insula (replicating Masten et al. (2013); Masten et al. (2010)) and possibly dACC given its role in experiencing social exclusion (Eisenberger et al., 2003). Finally, we expected participants would show elevated responses in mPFC, TPJ and NAcc (Lieberman & Eisenberger, 2009; Will et al., 2015) during acts of prosocial behavior (tossing the ball to the excluded player compared to tossing the ball to players who were not excluded).

Materials and methods

Participants

The final sample consisted of 23 healthy female participants of 18 and 19 years old (*M* = 19.08, *SD* = .48). One additional participant was excluded due to a technical error when collecting the MRI data. Participants were recruited through local advertisements. All participants were screened for MRI contra indications and psychopathology using a telephone interview before the scanning session. This study was approved by the Commission Medical Ethics of the Leiden University Medical Center. Written informed consent was obtained from all participants prior to the scan session. Participants received €30 for participation in a larger set of studies.

Experimental design

The Prosocial Cyberball game was an adapted version of the game used by Riem et al. (2013). The participant was depicted by a classical Cyberball figure (Williams et al., 2000) at the bottom of the screen. The other three figures portraying the three other players in the game were positioned to the left and right of the screen center, and at the top of the screen (see Fig 1). Participants were told that they would play a computer game in which players toss balls to each other, and were asked to imagine that they were playing the game with other individuals. Various studies have shown that knowingly playing a game against a computer can also lead to feelings of exclusion. For example, no differences were found between conditions in which participants merely imagine that they are playing with others or believe that other players are really present (Zadro, Williams, & Richardson, 2004). Therefore, we used the manipulation of imagining playing with others. This is a powerful manipulation in research on gaming (Konijn, Bijvank, & Bushman, 2007).

During the game, participants were instructed to toss the ball to the other players using a button box attached to their legs. The game consisted of two blocks. The first block was a fair situation, consisting of 120 trials. During this fair block all players received the ball an equal number of times. The second block was an exclusion situation, consisting of 168 trials. During this exclusion block, the player at the top of the screen (player 4) was excluded by the players positioned on the left and right of the screen center (players 1 and 3). Jitter was added at the end of each ball toss and ranged between 1000-2000 ms in steps of 500 ms.

Prosocial behavior during the game was measured by the ratio of tosses from the participant to the excluded player. This was calculated by dividing the number of tosses from the participant to the excluded player by the total number of tosses from the participant to any of the players. We expected to find a toss ratio of .33 from the participant for each player during the inclusion block, and a toss ratio larger than .33 from the participant to the excluded player during the exclusion block (based on Riem et al. (2013)). A toss ratio larger than .33 (relative to tosses to the other players) would indicate compensation for the exclusion by the left and right player.

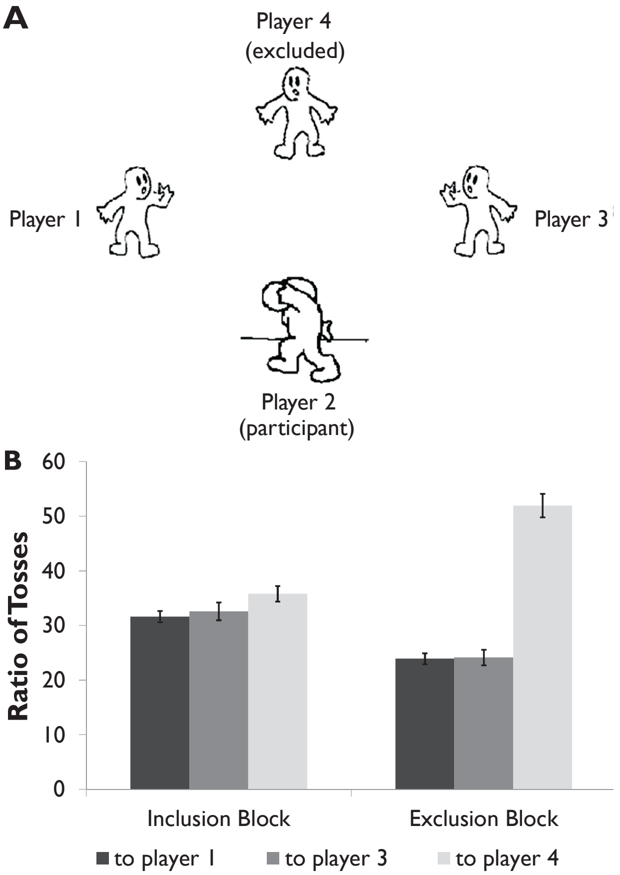


***Figure 1. (A)**: Example of stimulus display (participant numbers are shown for illustration purposes but were not presented to the participant), **(B)**: Ratio of tosses from the participant to the other three players in the inclusion and exclusion block.*

Questionnaire

A Dutch version (De Corte et al., 2007) of the Interpersonal Reactivity Index (IRI; Davis, 1983) was used to assess empathy. The IRI is a widely used self-report measure of empathy with 4 subscales that assess perspective taking (e.g. "Before criticizing somebody, I try to imagine how I would feel if I were in their place", 7 items, α = .73), empathic fantasy (e.g. "I really get involved with the feelings of the characters in a novel", 7 items, α = .86), empathic concern (e.g. "I am often quite touched by things that I see happen", 7 items, α = .86), and personal distress (e.g. “I sometimes feel helpless when I am in the middle of a very emotional situation”, 7 items, $\alpha = .63$). Deleting one of the items (“I sometimes feel helpless when I am in the middle of a very emotional situation”) from the subscale personal distress yielded $\alpha = .69$ after standardizing the remaining 6 items. All items can be answered on a five-point scale (0 = does not describe me well to 4 = describes me very well). On all subscales, high scores indicate higher levels of empathy.

Procedure

Participants received explanations regarding the procedure of an fMRI scan and the Prosocial Cyberball game. After these explanations, participants performed five practice trials of the Prosocial Cyberball game. Directly after the scanning session participants were administered a pen-and-paper version of the IRI questionnaire.

MRI data acquisition

Scans were made with a 3 Tesla Philips scanner, using a standard whole-head coil. The functional scans were acquired using a T2*-weighted echo-planar imaging (EPI). The first two volumes were discarded to allow for equilibration of T1 saturation effects (TR = 2.2 s, TE = 30 ms, sequential acquisition, 38 slices of 2.75 mm, field of view 220 mm, 80 × 80 matrix, in-plane resolution 2.75 mm). A high-resolution 3D T1-FFE scan for anatomical reference was obtained (TR = 9.760 ms; TE = 4.59 ms, flip angle = 8°, 140 slices, 0.875 × 0.875 × 1.2 mm3 voxels, FOV = 224 × 168 × 177 mm3). After the functional runs, a high resolution 3D T1-weighted anatomical image was collected (TR = 9.751 ms, TE = 4.59 ms, flip angle = 8°, 140 slices, 0.875 mm × 0.875 mm × 1.2 mm, and FOV = 224.000 × 168.000 × 177.333). Visual stimuli were shown on a screen that was attached in the magnet bore. Participants could see the stimuli via a mirror attached to the head coil. Head movement was restricted by using foam inserts inside the coil.

fMRI data analysis

All data were analyzed with SPM8 (Wellcome Department of Cognitive Neurology, London). Images were corrected for differences in rigid body motion. Structural and functional volumes were spatially normalized to T1 templates. Translational movement parameters never exceeded 1 voxel (3 mm) in any direction for any participant or scan. The normalization algorithm used a 12-parameter affine transform together with a nonlinear transformation involving cosine basis functions and resampled the volumes to 3 mm cubic voxels. Templates were based on the MNI305 stereotaxic space (Cocosco, Kollokian, Kwan, & Evans, 1997). Functional volumes were spatially smoothed with an 6 mm FWHM isotropic Gaussian kernel.

The start of each ball toss was modeled separately using a zero duration event (see Will et al. (2015)). The modeled events were separated in three overall contexts, which distinguished between the participant a) observing the other players toss to each other ("Observed Exclusion"), b) tossing the ball ("Ball Tossing"), or c) receiving the ball ("Ball Receiving"). These conditions were separated to control for confounding factors such as motor actions or motor preparation. As can be seen in Fig 1A, the participant was referred to as player 2, the excluders were referred to as players 1 and 3, and the excluded player was referred to as player 4.

The "Observed Exclusion" context in which the participant observed others playing were separated in "Observed Excluding" (player 1 to player 3 or vice versa) and "Connecting" (excluded player 4 to other players 1 and 3). The "Ball Tossing" context in which the participant was tossing the ball was separated in "Compensating" (participant to excluded player 4) and "Tossing" (participant to player 1 and 3 combined). Finally, the "Ball Receiving" context in which the participant received the ball was separated in "From Excluder" (players 1 and 3 to participant) and "From Excluded" (excluded player 4 to participant). The condition in which the other players (1 and 3) tossed the ball to the excluded player (4) was modeled separately but was not further analyzed, because this event occurred only once in the exclusion game. Therefore three separate contexts with a total of six conditions were used in the analyses. All events were time-locked to the moment of the start of the ball toss. The trial functions were used as covariates in a general linear model; along with a basic set of cosine functions that high-pass filtered the data. The least-squares parameter estimates of height of the best-fitting canonical HRF for each condition were used in pair-wise contrasts. The resulting contrast images, computed on a subject-by-subject basis, were submitted to group analyses.

Region of interest analyses

To test neural correlates of observed exclusion and prosocial behavior in the a priori defined brain regions, region-of-interest (ROI) analyses were performed with the Marsbar toolbox in SPM8 (Brett, Anton, Valabregue, & Poline, 2002). ROIs were selected based on previous studies showing associations with observing social exclusion (bilateral insula and dACC; Meyer et al., 2013), compensating for social exclusion (mPFC and TPJ; Moor et al., 2012; Will et al., 2015), and prosocial behavior (NAcc; Harbaugh et al., 2007; Lieberman & Eisenberger, 2009). For dACC, a template based on a large scale reverse-inference analysis on social pain was used (Lieberman & Eisenberger, 2015). The website "Neurosynth" (http://www.neurosynth.org) was used to extract independent templates for bilateral insula, mPFC, and bilateral TPJ. A mask for bilateral insula was created with the search term 'rejection' (retrieved on April 30th, 2015), using forward inference with a standard threshold of $Z > 2.3$. Masks for mPFC and bilateral TPJ were created with the search term 'mentalizing' (retrieved on April 24th, 2015), using forward inference with a standard threshold of $Z > 2.3$. The resulting templates for bilateral insula and mPFC were then masked with anatomical masks for these areas because they were connected with other regions. An anatomical mask of the left and right NAcc was extracted from the Harvard-Oxford subcortical atlas, with the threshold at 40%. The center of mass for each ROI, as well as the volume of each ROI is presented in Table 1. Results from these ROIs were compared with repeated-measures ANOVAs for the three separate contexts: Observed Exclusion (Observed Excluding vs Connecting), Ball Tossing (Compensating vs Tossing), and Ball Receiving (From Excluders vs From Excluded). The context Ball Receiving is reported for completeness, but we had no a priori hypotheses about this condition.

Whole brain analyses

To investigate neural responses on prosocial behavior across the brain we calculated the following contrasts, focusing on the exclusion game specifically. These analyses may reveal activity in brain regions other than the a priori selected areas. We tested the neural response to observing exclusion, with the contrast: Observed Excluding > Connecting (and the reversed contrast). We also tested the neural response to tossing prosocially to the excluded player, with the contrast: Compensating > Tossing (and the reversed contrast). Task-related responses were considered significant if they exceeded a cluster-corrected threshold of $p < .05$ FDR-corrected, at an initial threshold of $p < .005$ (Woo, Krishnan, & Wager, 2014).

Table 1. Coordinates and volumes of Regions of Interests that were extracted from Neurosynth and anatomical atlases (see text for details).

			Center of Mass		
Name	L/R	Volume (mm)	X	Y	Z
dACC		8880	1	15	30
NAcc	L	712	-10	12	-7
NAcc	R	696	10	13	-7
Insula	L	1352	-34	18	-1
Insula	R	1064	36	24	-1
mPFC		7304	-1	54	24
TPJ	L	7024	-50	-58	25
TPJ	R	7160	52	-55	23

Results

Behavioral results

To examine whether the participants compensated for the exclusion behavior by players 1 and 3 by tossing more balls to the excluded player (player 4), a repeated measures ANOVA with two within-subject factors was performed. Ratio of ball tosses from the participant to the other three players was the dependent variable (hereafter referred to as “player”), and play block (fair or exclusion) was the within-subject variable. In case the spherificity assumption was violated, we applied Greenhouse-Geisser corrections. The analysis resulted in a main effect of player ($F(2, 44) = 35.02$, $p < .001$, $\eta_p^2 = 0.69$), and a significant block*player interaction ($F(2, 44) = 50.92$, $p < .001$, $\eta_p^2 = 0.77$). No significant differences in ball tosses in the fair block were found ($F(1, 22) = 1$, $p = .33$). Post hoc contrasts revealed that in the exclusion block there was no significant differences in ratio of ball tosses to player 1 and 3 (*M* = 27.76, *SD* = .64, and *M* = 28.36, *SD* = 1.39, respectively). However, a significant difference was found in tosses to player 1 compared to player 4 ($F(1, 22) = 52.64$, $p < .001$, $\eta_p^2 = 0.77$), and between tosses to player 3 compared to player 4 ($F(1, 22) = 78.16$, $p < .001$, $\eta_p^2 = 0.85$; see Fig 1B). These findings show that participants compensated by tossing the ball more often to the excluded player.

In order to check whether behavior during the Prosocial Cyberball game was associated with self-reported empathy, we correlated the results of the IRI subscales with the ratio of ball tosses to the excluded player. There were no significant correlations with these subscales (see Table 2).

Table 2. Correlations between behavior during the exclusion game and self-reported empathy.

		M (*SD*)	PT	FS	EC	PD	To player 1	To player 3	To player 4
	PT	16.91 (4.17)							
	FS	19.73 (5.39)	0.32						
	EC	19.96 (5.64)	0.53*	.53**					
	PD	12.87 (4.13)	-0.37	0.23	0.11				
Ratio of tosses during PCG	To player 1	23.90 (4.86)	0.02	0.14	0.36	0.29			
	To player 3	24.14 (6.78)	0.19	-0.06	0.35	0.16	.57**		
	To player 4	51.96 (10.35)	-0.13	0.03	-0.4	-0.24	-.84**	-.92**	

**p < .05, *p < .01, PT = Perspective Taking, FS = Fantasy Scale, EC = Empathic Concern, PD = Personal Distress, PCG = Prosocial Cyberball Game*

ROI analysis

We performed region of interest analyses for insula, dACC, mPFC, TPJ,and NAcc (Fig 2) for the three separate contexts: Observed Exclusion, Ball Tossing, and Ball Receiving. Repeated measures ANOVAs were performed for each region and for each of the three contexts separately.

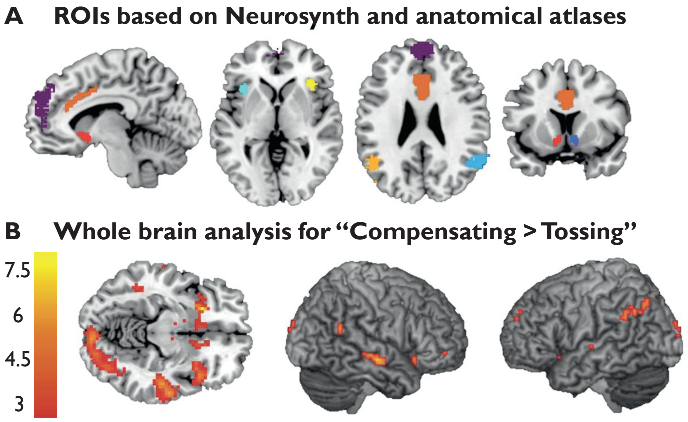


Figure 2. *Representation of **(A)** Independently selected ROIs: dACC (dark orange, most left picture), mPFC (purple, most left picture) bilateral insula (cyan and yellow, second picture to the left), bilateral TPJ (orange and blue, second picture to the right), and bilateral NAcc (red and dark blue, most right picture). See text for explanation of the selection procedure. See Table 1 for coordinates of center of mass. **(B)** Whole brain results for the contrast "Compensating > Tossing", for a cluster-corrected threshold of p < .05 FDR-corrected, 101 contiguous voxels, at an initial threshold of p < .005.*

Observed Exclusion

When participants were not tossing or receiving the ball, but were merely observing the other players, we compared Observed Excluding (players 1 and 3 toss to each other) with Connecting (player 4 tosses to player 1 or 3). We found significant differences between Observed Excluding and Connecting for left insula ($F(1, 22) = 10.24$, $p < .005$, $\eta_p^2 = 0.32$) and right insula ($F(1, 22) = 10.74$, $p < .005$, $\eta_p^2 = 0.33$), with more activation during Observed

Excluding than during Connecting (see Fig 3A). No condition effects were found for dACC, bilateral TPJ, mPFC and NAcc.

Ball Tossing

We tested the effects of compensating by tossing to player 4 versus tossing to player 1/3. We found a significant difference between Compensating and Tossing for left insula ($F(1, 22) = 7.74$, $p < .05$, $\eta_p^2 = 0.26$), right insula ($F(1, 22) = 7.35$, $p < .05$, $\eta_p^2 = 0.25$), left TPJ ($F(1, 22) = 6.72$, $p < .05$, $\eta_p^2 = 0.23$), right TPJ ($F(1, 22) = 6.90$, $p < .05$, $\eta_p^2 = 0.24$), left NAcc ($F(1, 22) = 5.22$, $p < .05$, $\eta_p^2 = 0.19$), and right NAcc ($F(1, 22) = 8.43$, $p < .01$, $\eta_p^2 = 0.28$) with more activation for Compensating than for Tossing (see Fig 3B). No effects were found for dACC and mPFC.

Ball Receiving

Finally, we tested whether there were differences between receiving the ball from excluding players 1 and 3 or from excluded player 4. For the left TPJ, we found a significant difference between receiving tosses From Excluders and From Excluded ($F(1, 22) = 5.45$, $p < .05$, $\eta_p^2 = 0.199$; see Fig 3C), with more activation during receiving from excluded than receiving from excluders. The other regions showed no condition effects.

Correlations with performance and self-reported empathy

None of the correlations between the parameter estimates for the three contexts and behavior during the Prosocial Cyberball Game were significant. In addition, none of the correlations between the parameter estimates for the three contexts and self-reported empathy were significant.

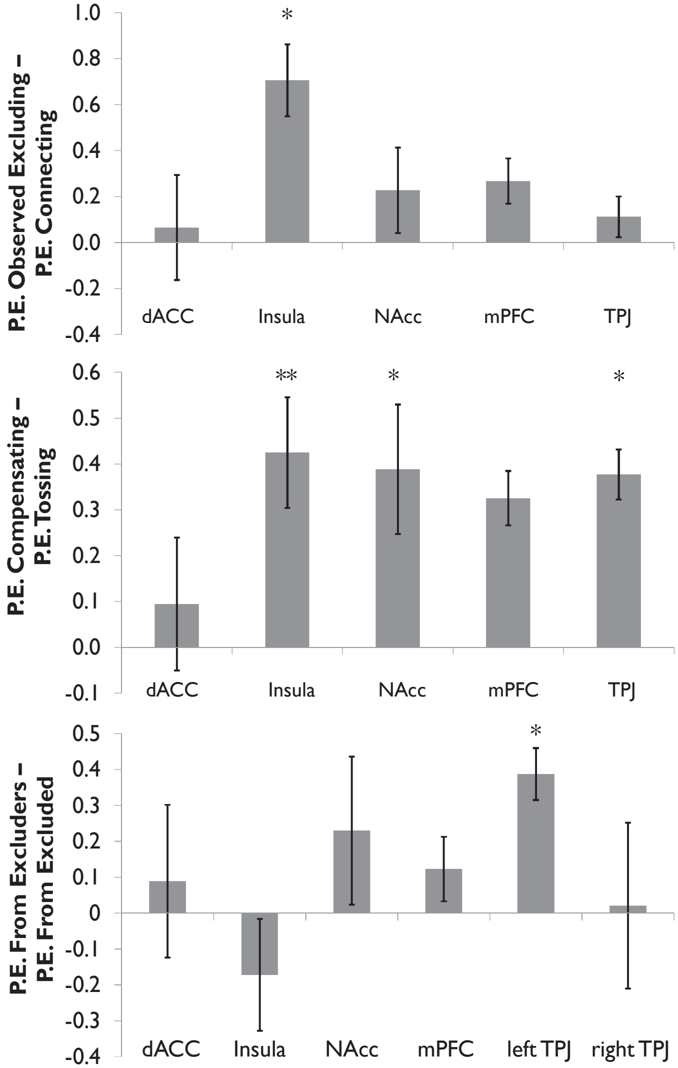


Figure 3. *Difference scores of activity in ROIs for the three condition contrasts. P.E. = parameter estimates. One asterisk (*) indicates p < .05 and two asterisks (**) indicate p < .01. Error bars represent Standard Errors (SE) of the mean. In case no significant differences were found between hemispheres, findings are presented collapsed across left and right lateralized areas.* ***(A)****: Difference score of activity in the ROIs for Observed Excluding – Connecting.* ***(B)****: Difference score of activity in the ROIs for Compensating - Tossing.* ***(C)****: Difference score of activity in the ROIs for receiving the ball From Excluders – receiving the ball From Excluded.*

Whole brain analysis

Exclusion game: Observed Exclusion.

To test for the neural correlates of observed exclusion, we tested neural correlates when participants were observing the others play. The contrast "Observed Excluding > Connecting" did not result in activation at the FDR cluster-corrected threshold.

Table 3. Whole brain table for neural activation for the contrast Compensating in the exclusion block > Tossing in the exclusion block (cluster corrected threshold of *p* < .05 FDR-corrected, at an initial threshold of *p* < .005).

			MNI coordinates		
Name	Voxels	T-value	X	Y	Z
Left insula	136	7.63	-24	20	-11
		4.74	-15	17	-14
		4.5	-30	26	-2
Left NAcc		3.62	-6	17	-11
Right superior temporal gyrus	226	6.68	60	-16	-8
		5.48	48	-22	-2
		3.97	45	-13	-17
Left cuneus	566	5.97	-6	-97	16
Right cuneus		5.94	6	-91	-8
		5.38	12	-88	16
Right superior temporal gyrus	209	5.3	54	-52	22
Right supramarginal gyrus (assigned to IPC)		4.04	54	-37	31
Right middle temporal gyrus		3.88	60	-52	7
Left angular gyrus	275	4.94	-42	-70	46
Left supramarginal gyrus (assigned to IPC)		4.82	-54	-49	34
Left angular gyrus (assigned to IPC)		4.72	-45	-55	37
Right insula/Inferior frontal gyrus	101	4.92	42	17	-11
Right temporal lobe		3.31	51	11	-14
Right inferior frontal gyrus		2.85	42	29	-2

Exclusion game: Compensation

To test for neural correlates of prosocial behavior, we tested neural correlates when participants were tossing the ball using the contrast "Compensating > Tossing". This contrast resulted in increased activity in a network of brain regions that are part of the social brain network, including left and right TPJ, left and right insula and NAcc (Fig 2). Other regions that were active in this contrast are presented in Table 3.

Discussion

The goal of this study was to test the neural regions that are associated with prosocial behavior in a social exclusion game. Consistent with prior studies, observing exclusion was associated with increased activity in the bilateral insula (Masten et al., 2013; Masten et al., 2010; Masten et al., 2011; Meyer et al., 2013). Thus, also in a context when the participant actively participates in the game observing exclusion leads to insula activation. When participants could compensate for exclusion, they tossed the ball more often to the excluded player than to the other players. This was associated with additional activity in the TPJ, previously associated with perspective taking (Carter & Huettel, 2013; Will et al., 2015) and the NAcc, previously associated with the rewarding feeling of doing good (Harbaugh et al., 2007). The behavioral results of this study fit with a set of prior studies that have shown that humans tend to compensate for observed distress of others (Masten et al., 2010; Masten et al., 2011; Will et al., 2013). The interpretation of this effect is that the observation of distress in others causes feelings of personal distress as well, leading to a tendency to act and decrease the observed distress. This was highlighted further in a study that used an oxytocin manipulation (Riem et al., 2013). When participants received a dose of oxytocin before the start of the game, they showed more compensation behavior. These results support the hypothesis that individuals with more empathic feelings show more compensation behavior, as oxytocin is known for enhancing empathy (Bartz et al., 2010).

The neural measures resulted in two important findings. First, compensation of excluded others was associated with elevated activity in the TPJ. The TPJ has previously been implicated in mentalizing and perspective taking (Carter & Huettel, 2013). Therefore, the findings suggest that compensation behavior involves thinking about what another person is thinking and feeling. The effects of TPJ were found in independently selected ROIs, and effects were further found in whole brain analyses. Notably, mPFC did not show increased activity for observed exclusion or for prosocial compensation. This effect is surprising given that prior studies showed an increase in mPFC activity during observed exclusion compared to observed inclusion (Masten et al., 2010; Masten et al., 2011). Also, in most social reasoning studies mPFC and TPJ are concurrently activated (for a review, see Blakemore (2008)), and functional connectivity studies showed that these regions are functionally connected (Burnett & Blakemore, 2009). It remains unclear why mPFC was not observed in prosocial behavior in the current study. Prior studies found that this region was active during prosocial conditions (FeldmanHall et al., 2012; Waytz, Zaki, & Mitchell, 2012). Possibly, in the current study prosocial behavior resulted in less conflict between self- and other oriented motives, because prosocial behavior was non-costly. Future studies should unravel the role of mPFC in costly and non-costly prosocial behavior.

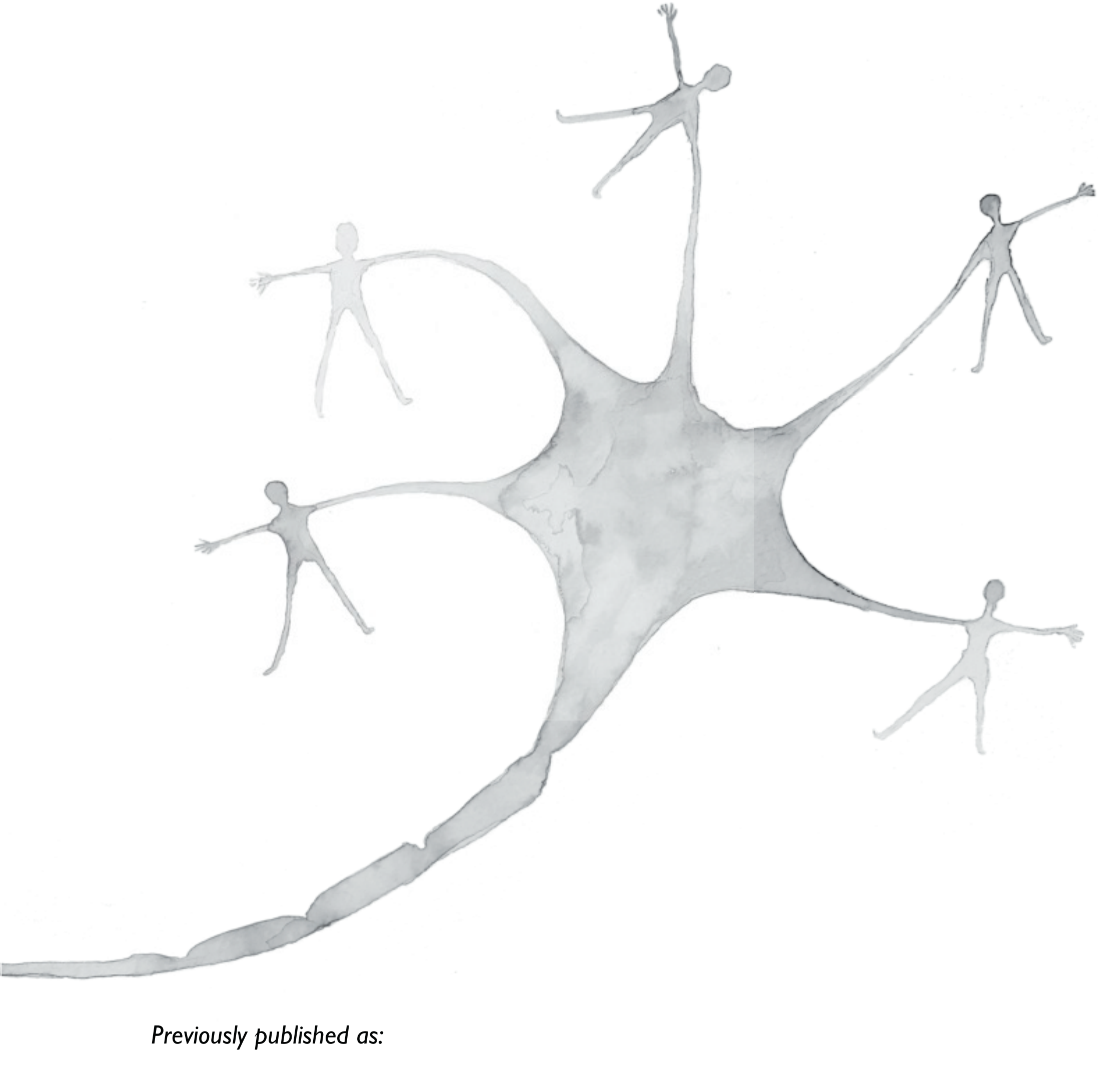
A second important finding was the increased activity in the NAcc for compensation of the excluded player, which was found in both the independently selected ROI analysis, and in the whole brain comparison. Several prior studies have reported that the NAcc is not only sensitive to primary rewards, such as juice or monetary rewards, but also to social rewards, such as being accepted or collaborating (for a review, see Lieberman and Eisenberger (2009)). The current study suggests that compensating others may be associated with a rewarding feeling as well. In a prior study by Harbaugh et al. (2007) it was found that the NAcc is more active during voluntary money transfers to charity than during mandatory transfers. The current findings fit well with this study showing that the NAcc is activated when participants can choose whether they compensate for exclusion behavior or not (see also Guroglu et al. (2014); Telzer (2016).

This study also had some limitations which should be addressed in future studies. First, the order of tasks did not allow us to test the contrast “observed exclusion > observed inclusion”, because the order of tasks was fixed (first inclusion, then exclusion). We therefore limited our analyses to the exclusion block. This may also account for the non-significant effects of dACC, given that prior studies found effects of exclusion blocks relative to inclusion blocks (Eisenberger et al., 2003). In future studies it will be important to present the task blocks interleaved, which would allow for a comparison of blocks of exclusion versus blocks of inclusion (Sebastian et al., 2011). Second, the participants were only women. Previous studies reported that neural responses to social interactions can differ for men and women (Singer et al., 2006). To increase power, we limited the participant selection to women. Future studies should test if the results are generalizable to men. Third, even though sufficient for testing main effects, the sample size was small for testing brain-behavior correlations. Interestingly, brain-behavior correlations were previously found in studies using similar sample sizes but other paradigms (Masten et al., 2013). In future studies, the question should be addressed whether prosocial compensation behavior is related to self-reported empathy, and whether this relationship can also be found between neural correlates of prosocial compensation behavior and self-reported empathy. Furthermore, to fully understand the relationship between prosocial behavior and self-reported empathy, multiple informants should be used to overcome limitations of self-reports and to better determine the empathic qualities of an individual (Hofstee, 1994).

To conclude, we confirmed that hypothesis that showing compensation behavior results in increased activity in a key area of the social brain network, which is the TPJ, and in addition showed that NAcc is recruited when showing compensation behavior. In contrast, observed exclusion only resulted in insula activity, suggesting separable contributions of these brain regions. This study demonstrated eligibility of a new paradigm to test prosocial behavior which dissociated between different components of understanding why and how individuals act prosocial. These results have important implications for studies that aim at individual differences in prosocial behavior, and they may suggest a useful tool for testing effects of prosocial interventions.

CHAPTER 3

The neural correlates of dealing with social exclusion in childhood



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Abstract

Observing social exclusion can be a distressing experience for children that can be followed by concerns for self-inclusion (self-concerns), as well as prosocial behavior to help others in distress (other-concerns). Indeed, behavioral studies have shown that observed social exclusion elicits prosocial compensating behavior in children, but motivations for the compensation of social exclusion are not well understood. To distinguish between self-concerns and other-concerns when observing social exclusion in childhood, participants (aged 7-10) played a four-player Prosocial Cyberball Game in which they could toss a ball to three other players. When one player was excluded by the two other players, the participant could compensate for this exclusion by tossing the ball more often to the excluded player. Using a three-sample replication (n = 18, n = 27, and n = 26) and meta-analysis design, we demonstrated consistent prosocial compensating behavior in children in response to observing social exclusion. On a neural level, we found activity in reward and salience related areas (striatum and dorsal anterior cingulate cortex (dACC)) when participants experienced inclusion, and activity in social perception related areas (orbitofrontal cortex) when participants experienced exclusion. In contrast, no condition specific neural effects were observed for prosocial compensating behavior. These findings suggest that in childhood observed social exclusion is associated with stronger neural activity for self-concern. This study aims to overcome some of the issues of replicability in developmental psychology and neuroscience by using a replication and meta-analysis design, showing consistent prosocial compensating behavior to the excluded player, and replicable neural correlates of experiencing exclusion and inclusion during middle childhood.

Introduction

Observing social exclusion occurs often in school-aged children and can be a distressing experience (Saylor et al., 2013). For example, when children observe that others are excluded from a game or social event, children may experience distress because they are concerned about their own inclusion, or they may feel the need to help the other person in distress, also referred to as prosocial behavior (Padilla-Walker & Carlo, 2014). Children show basic prosocial behavior from 18 months of age onwards (Warneken & Tomasello, 2006) and this behavior rapidly develops throughout childhood and adolescence when cognitive capacity and perspective taking skills continue to grow (Eisenberg et al., 2006; Güroğlu et al., 2014). However, the motivations for helping or compensation behavior remain largely unknown, possibly because these motives are difficult to unravel on the basis of behavior only. Neuroimaging may prove helpful to examine the different processes that take place when children observe social exclusion.

Social exclusion is often studied by using the Cyberball Game (Williams et al., 2000): a three player ball game where two virtual players no longer toss a ball to an excluded player, creating a situation of social exclusion. Although Cyberball is a computer game including virtual players, several studies have shown that both children and adolescents show more prosocial behavior in subsequent interactions towards individuals who have been excluded in this game, as indicated by helpful emails and money donations (Masten et al., 2010; Masten et al., 2011; Will et al., 2013). Recently a prosocial version of the paradigm was developed to examine concurrent compensating behavior when an individual is excluded (Riem et al., 2013). In the Prosocial Cyberball Game (PCG) participants can compensate for this exclusion by tossing the ball more often to the excluded player. Studies have shown that compensating behavior followed observed social exclusion towards the excluded player across childhood, adolescence and adulthood (Riem et al., 2013; van der Meulen, van IJzendoorn, & Crone, 2016; Vrijhof et al., 2016). Yet, it remains to be determined if children are most concerned about others when observing exclusion, or about self-inclusion and exclusion.

Neuroimaging research in adults revealed that simply observing another person being excluded is associated with increased activity in areas such as the dorsal anterior cingulate cortex (dACC) and bilateral insula (Masten et al., 2013; Meyer et al., 2013; Novembre et al., 2015). These regions are thought to play a role in social uncertainty and distress, and may be critically involved in experiencing concerns about self-exclusion (Cacioppo et al., 2013). Interestingly, previous studies have shown that the experience of being excluded yourself leads to feelings of decreased self-worth (Zadro et al., 2004), accompanied by an increase in activation of the dACC and bilateral insula (Cacioppo et al., 2013; Eisenberger et al., 2003; Rotge et al., 2015). Additionally, a recent study has added to this body of literature by postulating that co-activation in the dACC and bilateral insula is a measure of social inclusivity, and that activation in these two areas can therefore be found in both social exclusion and social inclusion contexts (Dalgleish et al., 2017).

In contrast, prosocial compensating behavior (i.e. compensating an excluded player) in the Prosocial Cyberball Game resulted in increased activation of the temporo-parietal junction (TPJ), nucleus accumbens (NAcc), and the bilateral insula (van der Meulen et al., 2016). The TPJ is an area previously associated with perspective taking (Carter & Huettel, 2013) whereas the NAcc is part of the reward network of the brain (Delgado, 2007; Lieberman & Eisenberger, 2009). Possibly, these regions play an important role in prosocial compensating behavior. These patterns of neural activity lead to the hypothesis that the Prosocial Cyberball Game might tap into two different processes: the experience or concern for possible self-exclusion and the compensation for exclusion of others. Experience of possible self-exclusion refers to the worry about own participation in the game, whereas compensation for exclusion is thought to reflect prosocial behavior.

The aim of the current study was to investigate the behavioral and neural correlates of reactions to observed social exclusion in middle childhood. Our target age was children in the age range 7-10 years because this is a critical age for forming intimate friendships and social connections (Buhrmester, 1990), but the neural reactions to observed social exclusion in this particular age range have not yet been studied. We used the Prosocial Cyberball Game (Riem et al., 2013) to study possible reactions to observed social exclusion, namely experience of possible self-exclusion and prosocial compensating behavior. Previous studies have called into question whether neuroimaging results survive Type I errors and may lead to too many false positives (Eklund, Nichols, & Knutsson, 2016). Moreover, recent projects have raised concerns about whether results from psychological experiments can be replicated (Open Science, 2015). Therefore, we used a replication approach including a pilot sample to generate hypotheses, a test sample to test these hypotheses, and a replication sample to confirm these findings. The test and replication sample consisted of co-twins because they are similar in many respects: this will optimize the chance for replication, and lack of replication cannot easily be ascribed to confounding or unmeasured differences between the two samples.

On a behavioral level we hypothesized that observing social exclusion would lead to prosocial compensating behavior (Riem et al., 2013; van der Meulen et al., 2016; Vrijhof et al., 2016). On a neural level we expected that both experiencing self-exclusion and self-inclusion would result in activity in dACC and bilateral insula (Cacioppo et al., 2013; Dalgleish et al., 2017; Eisenberger et al., 2003; Rotge et al., 2015). Furthermore, we expected that engaging in prosocial compensating behavior would lead to activity in dACC and bilateral insula (Masten et al., 2013; Masten et al., 2010) and TPJ, and NAcc, similar to what has been found in adults (van der Meulen et al., 2016). Although TPJ, dACC and bilateral insula show a sharp increase in cortical thickness during middle childhood (Mills et al., 2014; Pfeifer & Peake, 2012), not much is known about the functional role of these regions in observing social exclusion in middle childhood. The power of our experimental design suggests that the present set of studies is particularly sensitive to detecting brain-behavior relationships of higher socio-affective functions and their development in a developmental sample.

Materials and Methods

Participants

Three samples were recruited for this study: a pilot sample, a test sample and a replication sample. The pilot sample consisted of 20 children aged 7-10 years (*M* = 8.13 years, *SD* = .97, 50% male). This sample was composed of 9 opposite sex twin pairs and 2 singletons, recruited from an existing database at Leiden University. The test and replication sample consisted of 30 same sex twin pairs (*M* = 8.19 years, *SD* = .68, 46.7% male). Co-twins in the twin pairs were randomly divided over the test and replication sample upon inclusion, such that one child from each pair was placed in the test sample and one child was placed in the replication sample. These participants were recruited for the longitudinal twin study of the Leiden Consortium on Individual Development (L-CID). Families with twin children aged 7-8 years at the moment of inclusion were recruited from municipalities in the western region of the Netherlands, by sending invitations to participate to their home addresses (obtained through the municipal registries).

Some participants were excluded from analyses due to excessive head motion during the MRI session or because they did not finish the scanning session (two children from the pilot sample, three children from the test sample, and four from the replication sample). The final pilot sample consisted of 18 children (*M* = 8.15 years, *SD* = 1.06, 55.6% male), the final test sample of 27 children (*M* = 8.23 years, *SD* = 0.67, 40.7% male), and the final replication sample of 26 children (*M* = 8.21 years, *SD* = 0.71, 42.3% male). The three samples did not significantly differ in age ($F(2, 68) = .04$, $p = .96$) or gender ($X^2(2) = 1.08$, $p = .58$). All participants were screened for MRI contra indications, had normal (or corrected to normal) vision, were fluent in Dutch, and had no physical or psychological disorder that disabled their performance on the tasks. Written informed consent was obtained from both parents before the start of the study. Parents received €50 for the participation of their children, and all children received €3.50 and a goodie bag with small presents. The study was approved by the Dutch Central Committee on Research Involving Human Subjects.

Experimental Design

To measure reactions to observed social exclusion we used an experimental fMRI adapted version of the Prosocial Cyberball Game (PCG; Riem et al., 2013; van der Meulen et al., 2016; Vrijhof et al., 2016). In this game, participants see four classical Cyberball figures on the screen (Williams et al., 2000). The participant is represented by the figure at the bottom of the screen, and the three other figures are placed at the left, the right, and the top of the screen (see Figure 1A). Participants were told that they were going to play a computerized ball tossing game with three other players. No mention was made of exclusion, in order to avoid influencing their behavior. Thus, prosocial compensating is not confounded with varying biases between participants to follow the explicit or implicit experimenter suggestions for desirable behavior. Participants were asked to imagine that they were actually playing the game by thinking about the setting and the other players of the game. Previous studies have shown that there were no differences in reduced feelings of belonging and self-esteem between conditions where participants believed that other players were present, or merely imagined that other players were present (Zadro et al., 2004). Since imagining playing with others is a strong manipulation in research on gaming (Konijn et al., 2007) and does not rely on deception, we also used this manipulation for the PCG.

The game consisted of two parts: the Fair Game and the Unfair Game. During the first part (the Fair Game), the game was programmed to ensure that all four players received the ball an equal number of times. During the second part (the Unfair Game), either player 1 or player 3 tossed the ball only once to player 2 (at the top of the screen). After this initial toss, player 1 and player 3 no longer tossed the ball to player 2, thereby creating a situation of *observed* social exclusion for the participant. The participant could therefore choose to compensate for the exclusion by tossing more balls to excluded player 2, or to contribute to the exclusion by tossing more balls to players 1 and 3. The location of the excluded player was always the same for all participants (directly across the participant, at the top of the screen). In both the Fair Game and the Unfair Game, each trial consisted of a ball toss with a duration of 2000 ms. After each ball toss a jitter was added with a duration ranging from 1000-2000 ms in steps of 500 ms. The Fair Game consisted of 120 trials and was played on a laptop outside the MRI scanner. The Unfair Game consisted of 168 trials and was played in the MRI scanner, to enable collection of behavioral and MRI data during the task. During the Unfair Game, participants could indicate their response by pressing a button on a box attached to their right leg. The Unfair Game was presented in two separate parts to provide participants with a small rest period in between. During the entire game, the excluding players were referred to as Players 1 and 3 (on the left and right side of the screen respectively), the excluded player was referred to as Player 2, and the participant was referred to as "Participant" (see Figure 1A).

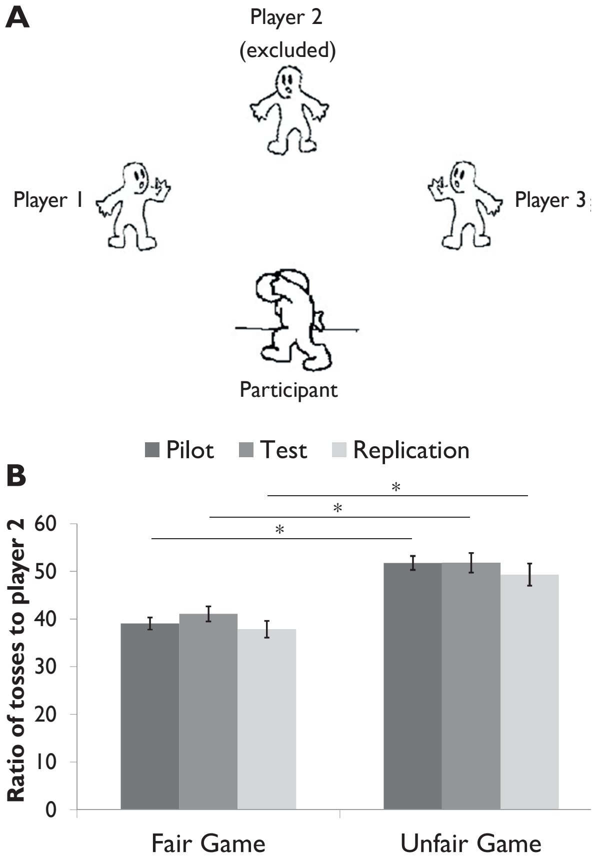


***Figure 1. (A)** Screenshot of Prosocial Cyberball Game. **(B)** Ratio of tosses of the participant to Player 2 in the PCG across the three samples.*

Procedure

Participants were given an extensive explanation and practice session in a mock scanner to familiarize them with the procedure of an MRI scan. All participants played the Fair Game of the PCG before the scanning session. Co-twins were then randomly assigned to either start with the scan session (and thus perform the Unfair Game of the PCG) or to start with other behavioral tasks that were part of the larger L-CID study. All twin pairs (from the pilot sample or from the test/replication sample) were randomly assigned to one of two procedures on the day of data collection.

MRI data acquisition

MRI scans were made with a Philips 3.0 Tesla scanner, using a standard whole-head coil. Data for the pilot sample were collected on a Philips Achieva TX MR, whereas data for the test and replication sample were collected on a Philips Ingenia MR. The functional scans were acquired using a T2*-weighted echo-planar imaging (EPI). The first two volumes were discarded to allow for equilibration of T1 saturation effects (TR = 2.2 s; TE = 30 ms; sequential acquisition, 37 slices; voxel size = 2.75 x 2.75 x 2.75 mm; Field of View = 220 x 220 x 112 mm). For the pilot sample the Field of View was 220 x 220 x 114.68 mm, with a sequential acquisition of 38 slices, and all other parameters were equal. After the functional runs, 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). For the pilot sample the TR was 9.76, the TE was 4.59, the voxel size was 0.875, and all other parameters were equal. Participants could see the stimuli projected on a screen via a mirror attached to the head coil. Foam inserts were used within the head coil to restrict head movement.

MRI data analyses

All data were analyzed with SPM8 (Wellcome Department of Cognitive Neurology, London). Images were corrected for slice timing acquisition and differences in rigid body motion. Functional volumes were spatially normalized to T1 templates. The normalization algorithm used a 12-parameter affine transform together with a nonlinear transformation involving cosine basis functions and resampled the volumes to 3 mm cubic voxels. Templates were based on the MNI305 stereotaxic space (Cocosco et al., 1997). Functional volumes were spatially smoothed with a 6 mm full width at half maximum (FWHM) isotropic Gaussian kernel. As a final step, the ArtRepair module (Mazaika, Hoeft, Glover, & Reiss, 2009) was used to address any head motions in the data. The threshold was set at 2 mm, and participants were excluded if more than 20% of the dynamics of the two functional runs were affected.

The start of each ball toss was modeled separately with a zero duration event. Since imaging data were collected during the Unfair Game but not during the Fair game, only the Unfair game was taken into account for these analyses. To study participant's experience of possible self-exclusion we differentiated between the participant receiving tosses from excluding Players 1 and 3 (“Experienced Inclusion”) versus the participant not receiving the ball from these players (“Experienced Exclusion”). To study participant’s compensation for observed exclusion of Player 2, we differentiated between the participant’s tossing to this excluded Player 2 (“Compensating”) versus his or her tosses to the excluding Players 1 and 3 (“Tossing to excluders”).

The trial functions were used as covariates in a general linear model; along with a basic set of cosine functions that high-pass filtered the data. The least-squares parameter estimates of height of the best-fitting canonical HRF for each condition were used in pair-wise contrasts. Motion regressors were included in the first level analysis. The resulting contrast images were computed on a subject-by-subject basis and then submitted to group analyses.

Whole brain analyses

We computed two different contrasts to study the various reactions to observed social exclusion. First, to investigate the neural response to being potentially excluded from the game by the other two players, we tested the contrast: *Experienced Inclusion > Experienced Exclusion* (and the reversed contrast). In accordance with the programming of the game, over the three samples the percentage of tosses from excluding Players 1 and 3 to the participant (M = 50.08, SD = .74) was comparable to the number of tosses from Players 1 and 3 to each other (M = 49.92, SD = .74). Over the three samples the percentage of tosses to the excluded player (M = 50.86, SD = 10.20) was comparable to the number of tosses to the two excluding players combined (M = 49.14, SD = 10.20). Second, to investigate the neural response to prosocial compensating behavior, we tested the contrast: *Compensating > Tossing to excluders* (and the reversed contrast). Significant task-related responses exceeded a cluster-corrected threshold of $p < .05$ FDR-corrected, with a primary threshold of $p < .005$ (Woo et al., 2014).

Region of interest analyses to test for replication effects

To further specify the effects of the whole brain analyses and to test for replication effects, functional ROIs were defined. We extracted functional clusters of activation from the whole brain contrasts in the pilot sample with the use of the MarsBar toolbox (Brett et al., 2002). Functional clusters that encompassed multiple anatomical regions were masked with anatomical templates from the MarsBar-AAL (Tzourio-Mazoyer et al., 2002) to separate the different anatomical regions. We then used the ROIs from the pilot sample to extract parameter estimates from the test sample. The same approach was used for the analysis of the results from the test sample to the replication sample.

Next, one-sided paired sample t-tests were used to test whether the activation in the first sample was significantly different between the conditions in the second sample. We corrected for multiple testing with a Bonferroni correction of alpha = .10, dependent on the number of extracted ROIs, because we were looking for replication of previously found results. Outlier scores (z-value < -3.29 or > 3.29) were winsorized (Tabachnick & Fidell, 2013).

To specifically explore the neural response during prosocial behavior across all three samples and to align our activation patterns with those found in adults, we used additional independent ROIs that were used in a study on prosocial neural responses in adults (see van der Meulen et al. (2016)). In the adult study, Neurosynth templates were used to create masks of the dorsal anterior cingulate cortex (dACC), bilateral insula, medial prefrontal cortex (mPFC), temporo-parietal junction (TPJ), and nucleus accumbens (NAcc). We used these masks to extract parameter estimates for the conditions "Compensating" and "Tossing" in all three samples. Combined effect sizes were computed with the Comprehensive Meta-Analysis (CMA) program (Borenstein, Rothstein, & Cohen, 2005).

Meta-analysis

We used an activation likelihood estimate (ALE) meta-analysis of whole brain results to test for commonalities across the three samples, for those contrasts that resulted in replicable effects. Given that the purpose of this meta-analysis was to test for commonalities among three samples that may not be observed in single studies, we used a less conservative threshold, which was then analyzed with a more stringent threshold at a meta-analytic level Coordinates from whole brain analyses conducted at a threshold of p < .001 uncorrected, 10 contiguous voxels, were entered in the Gingerale program (version 2.3.6, http://www.brainmap.org/ale/). We used a cluster correction of $p < .05$, with 1000 permutations and an initial primary voxel-wise threshold of $p < .001$.

Results

Behavioral results

The main behavioral outcome from the PCG is prosocial compensating behavior to Player 2, defined as an increase in ratio of tosses to Player 2 from the Fair game to the Unfair game. We calculated this ratio by dividing the number of tosses to Player 2 by the total number of tosses to all players (van der Meulen et al., 2016; Vrijhof et al., 2016). Paired t-tests were performed to study prosocial compensating behavior. Analyses that compare the first and second part of the Unfair Game (as these were presented as separate runs during the scan session) can be found in Supplement A.

First, in the pilot sample we found a significant difference in ratio of tosses to Player 2 in the Fair Game compared to the Unfair Game ($t(17) = -5.68$, $p = < .001$, $d = 2.20$). This finding was replicated in the test sample ($t(26) = -5.27$, $p < .001$, $d = 1.11$), and in the replication sample ($t(25) = -4.04$, $p < .001$, $d = 1.10$; see Table 1 for descriptives). Second, because children differed in their percentage of tosses to Player 2 in the Fair Game (see Figure 1B), we took these base-line differences into account by calculating a difference score between percentage of tosses to Player 2 in the Unfair Game minus the percentage of tosses to Player 2 in the Fair Game. Thus, for each participant a compensating score was calculated. We used an ANOVA to test whether there was a difference in compensating scores for the three samples, and found no significant difference ($F(2, 68) = .15$, $p = .86$). This shows that levels of prosocial compensating behavior were the same across the three samples during middle childhood.

Table 1. Descriptives of percentage of tosses of participant in Prosocial Cyberball Game. Data represent means (with standard deviations in parentheses).

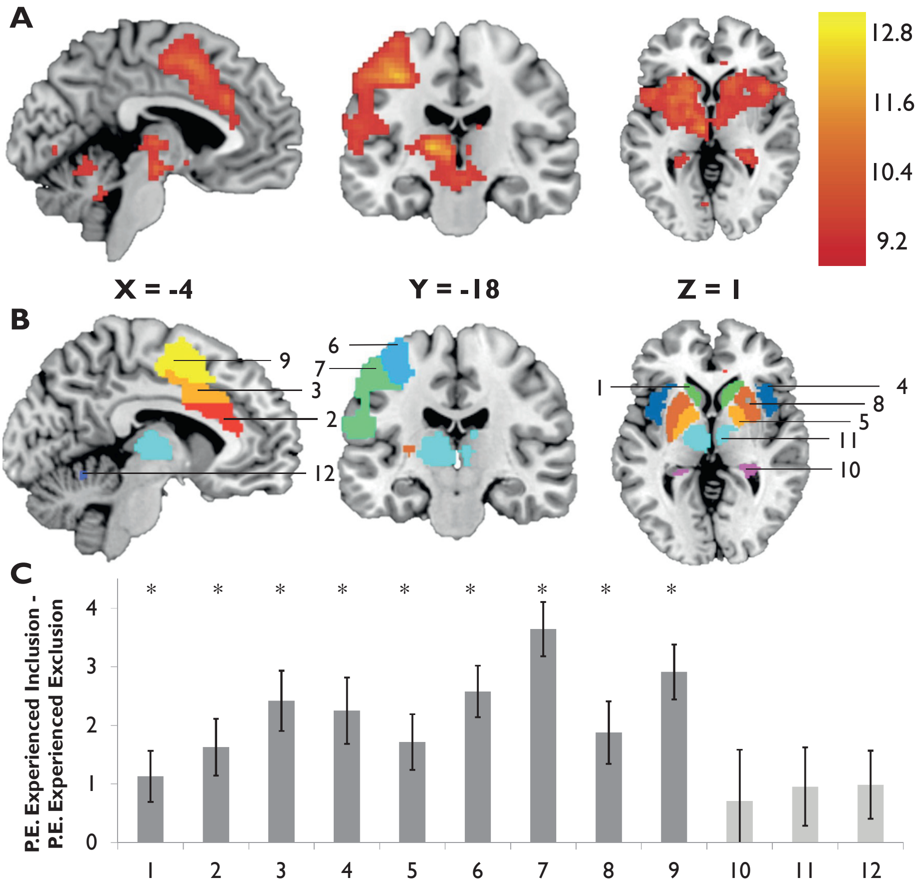
		Pilot	Test	Replication
	To player 1	30.47 (5.84)	30.52 (7.08)	31.21 (6.15)
Fair Game	To player 2	39.03 (5.34)	41.05 (8.14)	37.84 (9.03)
	To player 3	30.49 (5.51)	28.43 (6.37)	30.95 (6.57)
	To player 1	36.64 (6.22)	25.12 (7.66)	26.40 (7.10)
Unfair Game	To player 2	51.74 (6.19)	51.76 (10.75)	49.31 (11.87)
	To player 3	24.62 (6.92)	23.12 (6.58)	24.29 (8.54)

Neural reactions to Playing with Others

Experienced Inclusion > Experienced Exclusion

First, we tested the contrast Experienced Inclusion > Experienced Exclusion in the pilot sample with a whole brain analysis. The contrast was defined as receiving the ball from excluding Players 1 and 3 ("Experienced Inclusion") versus not receiving the ball from excluding Players 1 and 3 ("Experienced Exclusion"). The Experienced Inclusion > Experienced Exclusion analysis resulted in significant activation in several clusters that spanned medial prefrontal cortex (PFC; including pre-supplementary motor area (SMA), ACC), bilateral insula, bilateral striatum (including caudate, pallidum, putamen) and left pre- and postcentral gyrus (See Table 2 and Figure 2A). These were separated in 18 anatomically defined subclusters from which parameter estimates were extracted. When no significant differences were found between hemispheres, results were collapsed across left and right hemispheres. This resulted in a total of 12 regions that were analyzed in the test sample (see Figure 2B). Out of these 12 regions, bilateral caudate, insula, pallidum, and putamen, anterior and mid cingulum, left pre- and postcentral gyrus, and SMA, had significantly more activation for Experienced Inclusion than for Experienced Exclusion (all $p < .008$) in the test sample (see Figure 2C).

Next, we examined the contrast Experienced Inclusion > Experienced Exclusion in the test sample. This analysis resulted again in activation in several clusters that spanned medial PFC (including pre-SMA, ACC), bilateral insula, bilateral striatum (including caudate, pallidum, putamen) and left pre- and postcentral gyrus (See Table 2 and Figure 2D). These were separated in 14 anatomically defined subclusters from which parameter estimates were extracted. After collapsing results over hemispheres there were 10 regions included in the analysis for replication in the replication sample (see Figure 2E). Out of these 10 regions, bilateral insula and putamen, mid cingulum, left pre- and postcentral gyrus, and SMA had significantly more activation for Experienced Inclusion than for Experienced Exclusion (all $p < .01$) in the replication sample (see Figure 2F). For completeness the results of the contrast Experienced Inclusion > Experienced Exclusion in the replication sample are also reported in Table 2.



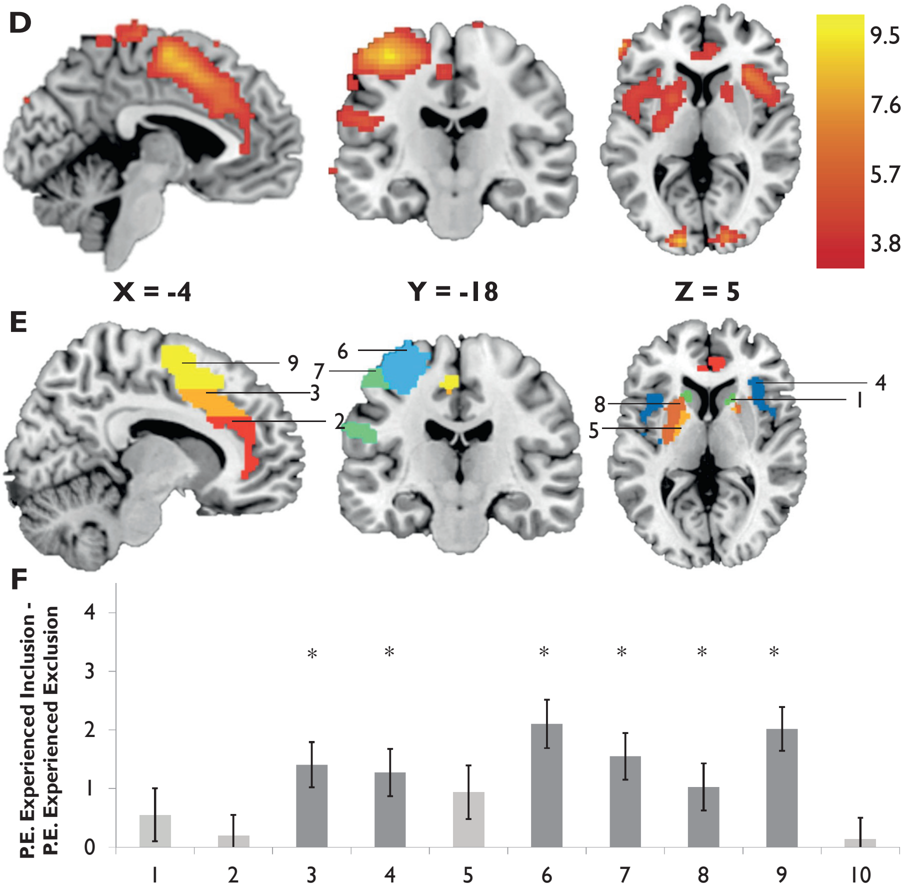


Figure 2. ***(A)*** *Whole brain results for the contrast "Experienced Inclusion > Experienced Exclusion" in the pilot sample.* ***(B)*** *Representation of anatomically separated ROI subclusters based on whole brain results: bilateral caudate (1), anterior cingulum (2), mid cingulum (3), bilateral insula (4), bilateral pallidum (5), left postcentral gyrus (6), left precentral gyrus (7), bilateral putamen (8), SMA (9), bilateral hippocampus (10), bilateral thalamus (11) and cerebellum (12).* ***(C)*** *Difference scores of activity in ROI subclusters in test sample.* ***(D)*** *Whole brain results for the contrast "Experienced Inclusion > Experienced Exclusion" in the test sample.* ***(E)*** *Representation of anatomically separated ROI subclusters based on whole brain results: bilateral caudate (1), anterior cingulum (2), mid cingulum (3), bilateral insula (4), bilateral pallidum (5), left postcentral gyrus (6), left precentral gyrus (7), bilateral putamen (8), SMA (9), and left middle frontal gyrus (10; not shown).* ***(F)*** *Difference scores of activity in ROI subclusters in replication sample. P.E. = parameter estimates. Error bars represent standard errors of the mean. Dark grey bars and asterisks (*) indicate replicated results.*

Table 2. Whole brain table for neural activation in the contrast "Experienced Inclusion > Experienced Exclusion" for the pilot and test sample, with a cluster corrected threshold of $p < .05$ FDR-corrected, at an initial threshold of $p < .005$.

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
PILOT					
Experienced Inclusion > Experienced Exclusion					
R Cerebellum	495	12.78	27	-55	-26
R Precuneus		9.75	15	-52	20
Cerebellar Vermis		7.54	5	-55	-11
L Thalamus	2740	11.94	-12	-16	7
		8.12	-12	-7	-2
L IFG		7.77	-51	8	7
L Postcentral Gyrus	2006	10.26	-36	-22	49
		8.22	-48	-22	49
L Anterior Cingulate Cortex		9.19	-12	23	31
TEST					
Experienced Inclusion > Experienced Exclusion					
L Postcentral Gyrus	2714	9.54	-45	-37	58
		8.58	-51	-25	58
L Precentral Gyrus		9.51	-39	-25	58
R Insula	393	5.97	33	23	7
		4.18	35	17	-8
R Putamen		3.53	21	8	-5
L Insula	877	5.56	-30	14	13
		4.52	-39	-7	22
L Pallidum		5.21	-21	2	-2
L Middle Frontal Gyrus	223	4.12	-33	47	28
		3.95	-35	47	37

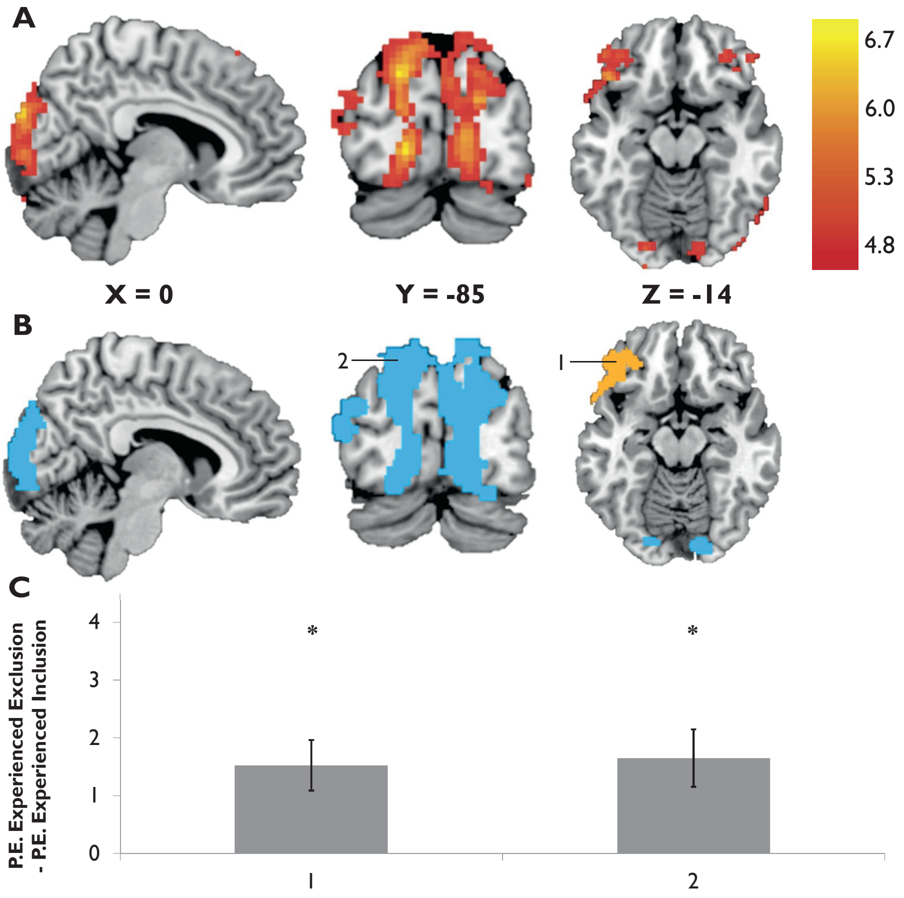
Table 2. Continued.

			MNI Coordinates		
	Voxels	T-Value	X	Y	Z
L Middle Frontal Gyrus		3.79	-45	41	31
REPLICATION					
Experienced Inclusion > Experienced Exclusion					
R SMA	1456	8.46	6	2	55
L Precentral Gyrus		7.46	-36	-28	61
L SMA		6.69	-6	2	49

Experienced Exclusion > Experienced Inclusion

Next, we tested the reversed contrast: Experienced Exclusion > Experienced Inclusion. In the pilot sample, this analysis resulted in two regions, a cluster in the left orbitofrontal lobe and a cluster in the occipital lobe (see Table 3 and Figure 3A). Two participants in the test sample had neural masks that did not completely cover these specific regions. Therefore one participant was excluded from analysis of activity in the left orbitofrontal lobe and one participant was excluded from analysis of activity in the left calcarine gyrus.

The analysis of parameter estimates extracted from the ROIs from this contrast and tested in the test sample showed that both regions were replicated in the test sample as showing greater activation for Experienced Exclusion than Experienced Inclusion (all $p < .005$; see Table 3 and Figure 3D). As a next step, the same whole brain analysis was performed in the test sample, which resulted in four regions: a cluster in the right paracentral lobe, two clusters in the occipital lobe, and a cluster in the left middle orbital gyrus. ROI values were extracted to test for replication in the replication sample. All four regions were replicated in the replication sample as showing greater activation for Experienced Exclusion than Experienced Inclusion (all $p < .001$). For completeness the results of the contrast Experienced Inclusion > Experienced Exclusion in the replication sample are also reported in Table 3.


A
6.7
6.0
5.3
4.8
X = 0
Y = -85
Z = -14
B
2
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P.E. Experienced Exclusion - P.E. Experienced Inclusion
4
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2

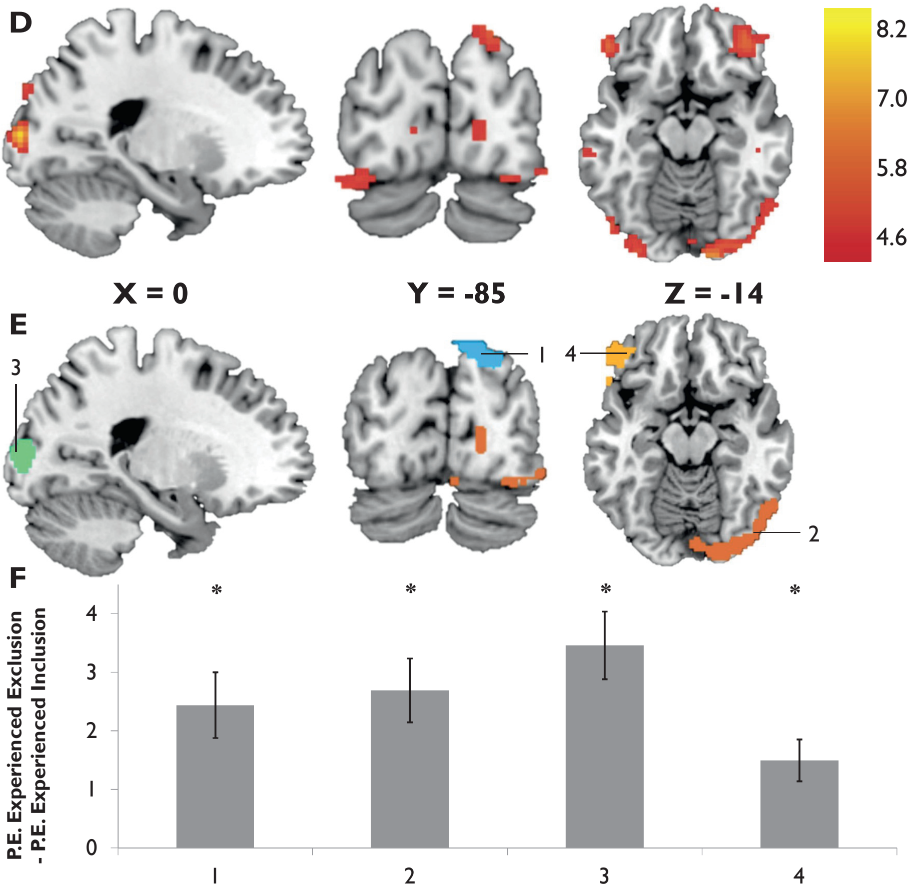


Figure 3. ***(A)*** *Whole brain results for the contrast "Experienced Exclusion > Experienced Inclusion" in the pilot sample.* ***(B)*** *Representation of anatomically separated ROI subclusters based on whole brain results: left IFG (1), and calcarine gyrus (2).* ***(C)*** *Difference scores of activity in ROI subclusters in the test sample.* ***(D)*** *Whole brain results for the contrast "Experienced Exclusion > Experienced Inclusion" in the test sample.* ***(E)*** *Representation of anatomically separated ROI subclusters based on whole brain results: right paracentral lobule (1), right cuneus (2), left middle occipital gyrus (3), and left middle orbital gyrus (4).* ***(F)*** *Difference scores of activity in ROI subclusters in the replication sample. P.E. = parameter estimates. Error bars represent standard errors of the mean. Dark grey bars and asterisks (*) indicate replicated results.*

Table 3. Whole brain table for neural activation in the contrasts "Experienced Exclusion > Experienced Inclusion" for the pilot and test sample, with a cluster corrected threshold of $p < .05$ FDR-corrected, at an initial threshold of $p < .005$.

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
PILOT					
Experienced Exclusion > Experienced Inclusion					
L Calcarine Gyrus	1422	6.79	-9	-91	-5
L Superior Occipital Gyrus		5.42	-18	-85	34
R Cuneus		5.41	9	-91	25
L Inferior Frontal Gyrus	264	6.75	-39	26	-17
		5.05	-18	17	-23
		4.77	-51	38	-8
TEST					
Experienced Exclusion > Experienced Inclusion					
R Cuneus	467	8.12	21	-91	10
R Lingual Gyrus		5.14	15	-97	-11
R Calcarine Gyrus		5.1	18	-97	-2
L Middle Occipital Gyrus	373	7.58	-18	-94	7
		4.8	-48	-79	-17
L Inferior Occipital Gyrus		4.47	-33	-94	-11
L Inferior Frontal Gyrus	326	5.96	-57	41	1
		5.21	-57	23	-11
		4.73	-51	41	-14
R Paracentral Lobe	543	4.85	-3	-58	76
		4.59	0	-25	73
R Precuneus		4.58	3	-73	54

Table 3. Continued.

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
REPLICATION					
Experienced Exclusion > Experienced Inclusion					
R Superior Occipital Gyrus	2758	7.34	24	-91	10
L Superior Occipital Gyrus		6.62	-15	-91	4
L Middle Occipital Gyrus		6.44	-27	-91	13
R Superior Frontal Gyrus	1721	7.23	21	32	64
L Superior Frontal Gyrus		6.87	-12	38	61
R Superior Frontal Gyrus		6.82	15	44	58
L Temporal Pole	1052	7.09	-57	17	-23
L Inferior Frontal Gyrus		6.63	-54	35	-17
		6.46	-57	26	-11
R Inferior Frontal Gyrus	387	5.06	33	29	-23
		5.03	30	38	-17
		4.7	42	29	-23

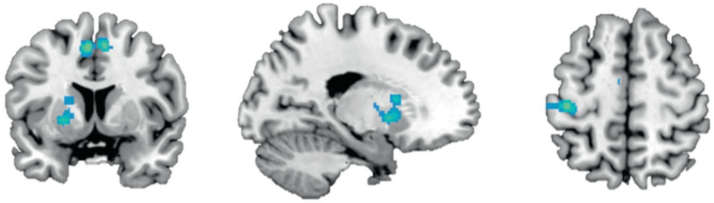
Whole brain ALE meta-analysis

To investigate common activation in the contrast Experienced Inclusion > Experienced Exclusion and its reversal, we performed a meta-analysis across the three samples. We found common activation in the contrast Experienced Inclusion > Experienced Exclusion in three clusters, namely the SMA/anterior cingulate, putamen/pallidum, and pre/postcentral gyrus (see Figure 4A, for coordinates see Table 3). For the reversed contrast, Experienced Exclusion > Experienced Inclusion, we found common activation in three clusters, including clusters in the occipital lobe and left orbitofrontal cortex (OFC; see Figure 4B, for coordinates see Table 4).

Table 4. Whole brain table for common activation across the three samples for the contrasts "Experienced Inclusion > Experienced Exclusion" and "Experienced Exclusion > Experienced Inclusion".

		MNI Coordinates		
Name	Voxels	X	Y	Z
Experienced Inclusion > Experienced Exclusion				
L SMA	3736	-6	6	50
		-8	10	44
		-6	-10	60
		-12	-10	60
R SMA		8	8	50
L Anterior Cingulate Cortex		-10	24	31
R Middle Cingulate Cortex		8	16	44
L Middle Cingulate Cortex		-8	16	38
L Putamen	1680	-22	4	-2
		-18	10	12
		-18	10	0
		-24	-6	10
L Pallidum		-18	-4	4
L Caudate		-16	16	4
L Precentral Gyrus	1064	-40	-24	58
L Postcentral Gyrus		-50	-24	58
		-48	-22	50
Experienced Exclusion > Experienced Inclusion				
R Cuneus	1176	18	-91	8
R Calcarine Gyrus		16	-80	10
L Orbitofrontal Cortex	1136	-50	42	-14
L Superior Occipital Gyrus	880	-16	-92	6

A Experienced Inclusion > Experienced Exclusion



B Experienced Exclusion > Experienced Inclusion

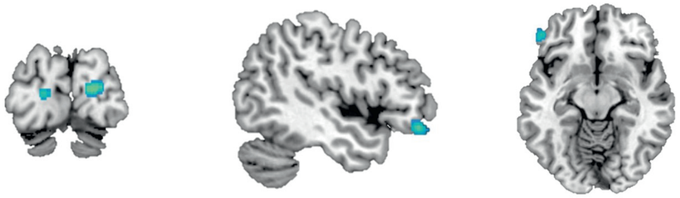


Figure 4. *Results from the whole brain ALE meta-analysis for the contrasts* ***(A)*** *Experienced Inclusion > Experienced Exclusion and* ***(B)*** *Experienced Exclusion > Experienced Inclusion.*

Neural reactions to Prosocial Compensating Behavior

Compensating versus Tossing to excluders

In the pilot sample, the contrast Compensating > Tossing to excluders resulted in one cluster in the occipital lobe (see Table 5). The reversed contrast resulted in another single cluster in the occipital lobe. ROIs were extracted for replication, but these regions were not replicated in the test sample. In the test sample, the contrast Compensating > Tossing to excluders and the reversed contrast did not result in significant activations. Because we found no significant activations in the test sample, we did not test this contrast in the replication sample.

Meta-analytic results for independent ROIs

The absence of neural effects for prosocial compensating behavior was unexpected considering the behavioral results and the results of previous studies on neural correlates of Cyberball (van der Meulen et al., 2016). Therefore, we performed a meta-analysis on pre-defined ROIs from an adult study (van der Meulen et al., 2016): the bilateral insula, left and right TPJ, and bilateral NAcc. Parameter estimates from these ROIs were extracted and combined in a meta-analysis. However, we found no significant pattern of activation during prosocial behavior across the three samples (see supplementary table S1).

Table 5. Whole brain table for neural activation in the contrast Compensating > Tossing to excluders (and reversed), with a cluster corrected threshold of $p < .05$ FDR-corrected, at an initial threshold of $p < .005$

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
PILOT					
Compensating > Tossing to excluders					
L Cuneus	149	5.42	-6	-94	16
		4.35	-5	-91	25
L Calcarine Gyrus		5.08	3	-94	13
Tossing to excluders > Compensating					
R Calcarine Gyrus	195	6.22	12	-76	7
R Lingual Gyrus		3.28	9	-58	1
		3.89	15	-54	-5

Relation with prosocial compensating behavior

Lastly, we were interested in whether activity in areas that were observed in the meta-analyses was related to prosocial compensating behavior. Therefore, we created spheres based on the coordinates of the clusters found in the meta-analyses. We chose coordinates for the ACC, putamen, pre-/postcentral gyrus, SMA in the “Experienced Inclusion > Experienced Exclusion” contrast, and coordinates for the OFC in the “Experienced Exclusion > Experienced Inclusion” contrast (see Table 3). Spheres were created with a diameter of 5 mm. The resulting spheres were then submitted to ROI analyses for each of the three samples, and resulting parameter estimates were correlated with prosocial compensating behavior (defined as the compensating score obtained in the PCG). In all three samples no significant associations were found between prosocial compensating behavior and parameter estimates from any of the ROIs.

Discussion

This study examined the neural correlates of observing social exclusion in a four-player Prosocial Cyberball Game during middle childhood. As expected, the exclusion of a fourth player by two others resulted in increased ball tossing by the participant to the excluded player. This is consistent with earlier findings of helping or compensating behavior in children who observed social exclusion of others (Vrijhof et al., 2016; Will et al., 2013). The behavior was robust across three samples. Furthermore, in a meta-analysis across the three samples there was increased activity in striatum and dACC when participants experienced inclusion themselves, and increased activity in orbitofrontal cortex when participants experienced exclusion, consistent with prior studies showing that these are important areas for the feelings of inclusion and exclusion in traditional Cyberball games (Lieberman & Eisenberger, 2009). However, contrary to our expectations, there were no neural regions that distinguished between compensating an excluded player and tossing the ball to the non-excluded players. The pattern of increased activity in social-affective brain regions as previously found in adults (van der Meulen et al., 2016) could not be confirmed in 7-10-year-old children, even when we used specific regions of interest in the social brain network or in a meta-analysis.

The strongest and most consistent findings were observed for the contrast experienced self-inclusion versus experienced self-exclusion. That is to say, experienced self-inclusion (receiving the ball from the two excluding players) was associated with increased activity in the striatum and the dACC in each of the three samples, and this was confirmed in a meta-analysis. These neural regions have also been consistently implicated in reward processing (Bhanji & Delgado, 2014; Delgado, 2007), and dACC activity specifically has been argued to signal evaluation and appraisal of an upcoming event (Shenhav, Cohen, & Botvinick, 2016). These findings may indicate that self-inclusion is important for children in ball tossing games. Indeed, prior studies showed that children who were not included by their peers reported feeling less happy and more angry (Saylor et al., 2013), and showed higher levels of cortisol, an indication of increased levels of stress (Gunnar, Sebanc, Tout, Donzella, & van Dulmen, 2003).

The reversed contrast, experienced self-exclusion (not receiving the ball from the two excluding players) was associated with activation in the orbitofrontal cortex. This region was previously observed in adults in a meta-analysis on social exclusion (Cacioppo et al., 2013), possibly indicating that this region is generally observed across children and adults when not being included. The orbitofrontal cortex is thought to play a role in managing social perceptions (Hughes & Beer, 2012). It should be noted that prior studies, including meta-analyses (Cacioppo et al., 2013), also pointed to the dACC and bilateral insula as important regions for exclusion, whereas in the current study the dACC was observed for inclusion. However, the role of the dACC and insula in exclusion has been debated, and possibly it is signaling the salience of an event (Menon & Uddin, 2010; Seeley et al., 2007) rather than specific activation for social events. Taken together, across three samples and confirmed by a meta-analysis, we observed consistent neural activation patterns for experienced self-inclusion and self-exclusion in 7-10-year-old children, validating this as a paradigm to investigate responses to a situation of social exclusion.

We found no evidence in the current study for neural regions that correlate with prosocial compensating behavior, that is to say, ball tossing to the excluded player versus ball tossing to the other players. This is surprising, because behaviorally there was a strong and consistent compensating pattern in all three samples. We previously observed in adults that bilateral insula, TPJ and NAcc were activated when tossing to an excluded player versus tossing to the other players (van der Meulen et al., 2016). However, previous studies that examined giving behavior in children and adolescents observed that children do not yet differentiate between intentions for giving (Güroğlu et al., 2009) and that activity in TPJ associated with intention understanding develops during adolescence (Güroğlu et al., 2011). Even though children as young as four years old understand the norms for fair distributions of goods, they only behave in accordance with those norms when they reach the age of eight (Smith et al., 2013). Furthermore, it is unclear when children's motivations for fair behavior shift from a desire to follow the norms to the understanding of someone else's needs. The current study cannot give a conclusive answer to this question because there was no comparison group with older participants. However, earlier research has indicated that activity in TPJ increases with age, especially for situations where perspective taking is required (Crone, 2013). Therefore, it would be interesting for future studies to test whether this developmental increase extends to other social brain regions, and whether this increase in activity can be related to changing motives for prosocial compensating behavior.

This study has significant strengths, such as the replication design that was used to test and replicate results from one sample to two other samples. The addition of a meta-analytic approach further confirmed our results. Furthermore, the current study is one of the first to investigate behavioral and neural correlates of prosocial compensating behavior in middle childhood. Nevertheless, there also were some limitations that should be addressed in future studies. First, the two processes studied (prosocial compensating behavior and experience of possible self-exclusion) are dependent on each other, as the participant first has to receive the ball from the excluders before they are able to engage in prosocial compensating behavior. This might provide a bias for the analysis used in this study although the number of tosses in each contrast was comparable. Second, the contrast used to study neural findings for prosocial compensating behavior (tossing to excluded player vs tossing to other players in the unfair situation) might not be the optimal situation to study these reactions. Ideally, a comparison similar to the difference score in the behavioral results would be made: a comparison in tossing to player 2 during the unfair situations versus tossing to player 2 during the fair situation. However, given that imaging data was not collected during the fair situation, we believe that we have chosen the best possible contrast to measure prosocial behavior, as it only includes behavior from the participant (tossing to excluded or to other players) and is therefore comparable in for example motion and time-one-task confounds. Third, the test and replication sample were not completely independent from each other. For these two samples same-sex co-twins were randomly assigned to the test or replication sample. Therefore, the results could be more similar for the test and replication sample than for the pilot sample. In fact, the replication step from test to replication sample was optimized in that the two samples were perfectly matched on age, gender, family background, and in about half of the cases even on genetic make-up. A randomized co-twin design leaves much less room for alternative interpretations in case of non-replication. Finally, the sample size of our three samples was too small to examine individual differences in motives for prosocial compensating behavior. This would be an important step in investigating the underlying reasons for children to engage in prosocial behavior in the Prosocial Cyberball Game, and therefore this question should be addressed in a larger sample.

In conclusion, the current study confirmed the hypothesis that children ages 7-10-years show prosocial compensating behavior in a relatively new paradigm in children: the Prosocial Cyberball Game. Interestingly, we found no strong evidence for specific neural activity related to prosocial compensating behavior towards the excluded player, but robust evidence was found for neural contributions to feelings of self-inclusion and –exclusion. The relation between prosocial compensating behavior and neural activity during self-inclusion and –exclusion is not yet clear, but possibly these findings highlight the switch from self to other motivations to engage in prosocial compensating behavior in late childhood and emerging adolescence. Alternatively, there may be important individual differences between children that emerge in larger samples. These hypotheses will be tested in a future longitudinal design, as these children will be followed over several years. Here, we presented a new approach to the hotly debated issue of replicability in behavioral and neuroscience showing that answers might be dependent on specific contrasts and underlying neural mechanisms even within the same paradigm.

Supplementary Materials

Supplement A

Since the Unfair Game in the PCG was presented in two parts during the scanning session, we wanted to investigate whether there was a significant difference in behavior from the first to the second part. First, in the pilot sample we found a significant difference in ratio of tosses to Player 2 in the Fair Game compared to the first part of the Unfair Game ($t(17) = -3.27$, $p = .005$, $d = 1.32$) and compared to the second part of the Unfair Game ($t(17) = -7.32$, $p < .001$, $d = 2.44$). This finding was repeated in the test sample, where we also found a significant difference in ratio of tosses to excluded Player 2 in the Fair Game compared to the first part of the Unfair Game ($t(26) = -3.72$, $p = .001$, $d = .81$) and compared to the second part of the Unfair Game ($t(26) = -4.93$, $p < .001$, $d = 1.15$). These results were also replicated in the replication sample, where we again found a significant difference in ratio of tosses to excluded Player 2 in the Fair Game compared to the first part of the Unfair Game ($t(25) = -3.47$, $p = .002$, $d = .97$) and compared to the second part of the Unfair Game ($t(25) = -3.96$, $p = .001$, $d = 1.05$). The increase in ratio of tosses to Player 2 was between 8-10% for the first part of the Unfair Game, and between 13-16% for the second part of the Unfair Game across the three samples.

Second, because children differed in their percentage of tosses to Player 2 in the Fair Game, we took these base-line differences into account by calculating a difference score between percentage of tosses to Player 2 in the Unfair Game minus the percentage of tosses to Player 2 in the Fair Game (base-line). Next, we used a paired t-test to investigate whether the difference scores for the two parts of the Unfair Game were significantly different from each other. We found a significant difference between the difference scores for first and second part of the Unfair Game ($t(17) = -2.85$, $p = .01$, $d = .64$) in the pilot sample, but not in the test and replication sample ($p = .07$ and $p = .21$ respectively). Because of the high correlation in the pilot sample between percentage of tosses to Player 2 in the first and second part of the Unfair Game ($r = .57$) we decided to combine the two parts in each of the three samples by adding the difference score for the first and second part of the Unfair Game, divided by two. We used an ANOVA to test whether there was a difference in average compensating scores between the three samples, and found no significant difference

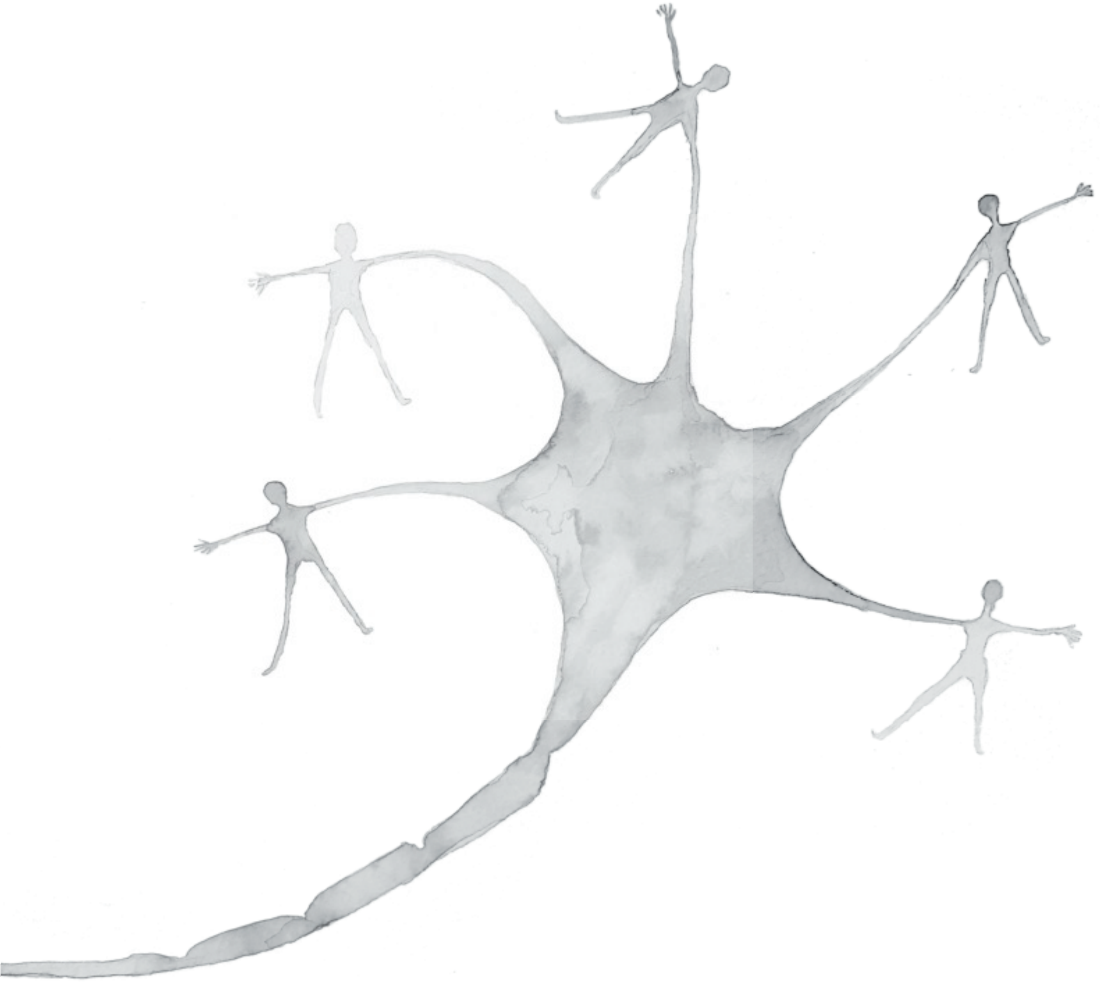
($F(2, 68) = .14$, $p = .87$). This shows that levels of compensating behavior were the same across the three samples.

Table S1. Results meta-analysis on parameter estimates from pre-defined ROIs for the conditions Compensating – Tossing to excluders.

		Hedge's g	***p*-value**
Insula	*Pilot*	-0.07	0.5
	Test	-0.15	0.42
	Replication	0.09	0.57
	Meta	-0.05	0.6
NAcc	*Pilot*	-0.19	0.17
	Test	0.06	0.67
	Replication	0.17	0.25
	meta	0.01	0.94
L TPJ	*Pilot*	0	0.98
	Test	0.05	0.72
	Replication	-0.09	0.47
	Meta	-0.02	0.78
R TPJ	*Pilot*	-0.1	0.56
	Test	0.01	0.96
	Replication	-0.05	0.69
	Meta	-0.05	0.58

CHAPTER 4

Heritability of neural reactions to social exclusion and prosocial compensation in middle childhood



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Abstract

Experiencing and observing social exclusion and inclusion, as well as prosocial behavior, are important aspects of social relationships in childhood. However, it is currently unknown to what extent these processes and their neural correlates differ in heritability. We investigated influences of genetics and environment on experiencing social exclusion and compensating for social exclusion of others with the Prosocial Cyberball Game using fMRI in a twin sample (aged 7-9; *n* = 500). Neuroimaging analyses (*n* = 283) revealed that experiencing possible self-exclusion resulted in activity in inferior frontal gyrus and medial prefrontal cortex, which was influenced by genetics and unique environment. Experiencing self-inclusion was associated with activity in anterior cingulate cortex, insula and striatum, but this was not significantly explained by genetics or shared environment. We found that children show prosocial compensating behavior when observing social exclusion. Prosocial compensating behavior was associated with activity in posterior cingulate cortex/precuneus, and showed unique environmental effects or measurement error at both behavioral and neural level. Together, these findings show that in children neural activation for experiencing possible self-exclusion and self-inclusion, and for displaying prosocial compensating behavior, is accounted for by unique environmental factors and measurement error, with a small genetic effect on possible self-exclusion.

Introduction

Social exclusion is a common event for school-aged children: in day-to-day interactions they either experience exclusion themselves, or they observe someone else being excluded. Experience of exclusion can lead to personal distress (Saylor et al., 2013), whereas the observation of someone else's exclusion often leads to prosocial compensating behavior (Masten et al., 2011), although in some cases individuals may also join in exclusion to follow group norms, possibly in order to prevent self-exclusion (Over & Carpenter, 2009). However, research to date remained inconclusive with respect to how experiencing exclusion and acting prosocially upon observed exclusion can be distinguished from each other in school-aged children, and whether these processes are differentially influenced by genetic and environmental factors. Earlier studies have indicated that sensitivity to experiencing social exclusion is influenced by personal experiences (Masten, Telzer, Fuligni, Lieberman, & Eisenberger, 2012), whereas prosocial compensating behavior is both influenced by genetics (Knafo-Noam et al., 2015; Knafo & Plomin, 2006) and by the environment (Menting, de Castro, & Matthys, 2013; Newton, Laible, Carlo, Steele, & McGinley, 2014). A better understanding of heritability of social exclusion sensitivity and prosocial compensating behavior in middle childhood might help us explain the underlying mechanisms and provides insights for future development of (school-based) interventions.

Both social exclusion and subsequent prosocial compensating behavior have previously been studied with the Cyberball Game (Masten et al., 2011; Will, van den Bos, Crone, & Güroğlu, 2013; Williams et al., 2000). A four-player adaptation of this paradigm was used to study the experience of social exclusion and prosocial compensating behavior in a situation of observed social exclusion (Tousignant et al., 2017; van der Meulen et al., 2017; van der Meulen et al., 2016). After an initial round of fair play, one player (not the participant) is excluded by the two other players. This manipulation allows the participant to either join in the exclusion or compensate for the exclusion by tossing more balls to the excluded player than to either of the two excluding players (i.e. prosocial compensating). In addition, the participant does not receive the ball for short periods of time from the two excluding players, which might lead to alternating feelings of worry about possible self-exclusion and relief about self-inclusion. Neural activation analyses in prior research revealed that experiencing alternating social exclusion was associated with increased activity in left inferior frontal gyrus (IFG) in children (van der Meulen et al., 2017). This finding reflects meta-analyses in adolescents and adults showing that the lateral orbitofrontal cortex (overlapping with the IFG) and subgenual anterior cingulate cortex (sgACC) are more active when experiencing social exclusion (Cacioppo et al., 2013; Rotge et al., 2015; Vijayakumar, Cheng, & Pfeifer, 2017), as is the amygdala (Eisenberger, Gable, & Lieberman, 2007). Interestingly, studies in which participants experienced exclusion during short intervals showed comparable results to studies examining social exclusion in more prolonged social rejection contexts. For example, it was found that the medial prefrontal cortex (mPFC) was activated during an extended period of exclusion in a classic Cyberball Game (Gunther Moor et al., 2012) as well as during short intervals of rejection in a social judgment task (Gunther Moor, van Leijenhorst, Rombouts, Crone, & Van der Molen, 2010). Moreover, studies also report that effects of social exclusion and rejection are sensitive to social experiences, such as a long-term history of social exclusion (Will et al., 2016) or childhood maltreatment (van Harmelen et al., 2014). Social inclusion, in contrast, was previously associated with activity in dorsal ACC (dACC) and the striatum (Davey et al., 2010; van der Meulen et al., 2017), which possibly signals the saliency and reward of this event (Menon & Uddin, 2010; Seeley et al., 2007).

A critical element of the four-player Prosocial Cyberball game is that it also allows for the study of prosocial compensating behavior towards an excluded player. Prior studies using the Prosocial Cyberball Game showed that both adults and children indeed engage in prosocial compensating behavior after observing prolonged social exclusion of a different player (Riem et al., 2013; Tousignant et al., 2017; van der Meulen et al., 2017; van der Meulen et al., 2016; Vrijhof et al., 2016). Interestingly, in adults, but not in children, prosocial compensating behavior was associated with increased activity in the temporo-parietal junction (TPJ; Tousignant et al., 2017; van der Meulen et al., 2016; Will et al., 2015), which is considered to be part of the social brain network (Blakemore, 2008; Frith & Frith, 2007). Moreover, in studies using social dilemma paradigms, it was also found that prosocial behavior was associated with increasing activity in the TPJ over the course of adolescence (Güroğlu et al., 2009, 2014; Güroğlu et al., 2011; Tousignant et al., 2017). Finally, prior studies also showed consistent involvement of the ACC-insula network when participants acted against their own social norms, which was independent of age (Güroğlu et al., 2011). These findings warrant further investigation of the neural regions and motives that children use when acting prosocially towards others.

An important, but understudied question concerns to what extent neural activity in these regions is sensitive to genetic and environmental influences. Earlier studies have focused on heritability of brain volume (Teeuw et al., 2018), brain connectivity (for review see Richmond, Johnson, Seal, Allen, and Whittle (2016)) and brain activity during cognitive tasks (for review see Jansen, Mous, White, Posthuma, and Polderman (2015)), and found significant influences of genetics. A prior study on heritability effects on neural correlates of social rejection showed small influences of genetics in middle childhood (Achterberg, van Duijvenvoorde, van der Meulen, Bakermans-Kranenburg, & Crone, 2018), but to our knowledge there are currently no studies that have directly investigated heritability effects on neural correlates of social inclusion and prosocial behavior. Therefore it remains an important question whether these processes are more sensitive to genetic or environmental influences.

This study therefore had two goals: I) To test the main contrasts and the brain-behavior relations of possible social exclusion, inclusion, and prosocial compensating. II) To examine the heritability of social processes in brain regions that are involved in possible self-exclusion, self-inclusion, and prosocial compensating. Therefore we investigated the genetic versus environmental influences on brain activity in middle childhood using a twin design. First, we expected that experiencing self-exclusion would be associated with activation in IFG and sgACC (Cacioppo et al., 2013; Vijayakumar et al., 2017), as well as mPFC (Gunther Moor et al., 2012) and amygdala (Eisenberger et al., 2007), whereas experiencing inclusion was expected to lead to activation in bilateral insula/ACC (Menon & Uddin, 2010; Seeley et al., 2007) and the striatum (Van der Meulen et al., 2016). Second, we expected that children would show prosocial behavior in situations of observed exclusion (Masten et al., 2011). Third, we expected that social brain areas (mPFC, precuneus, TPJ and STS) would be be activated when acting prosocially (Guroglu et al., 2014; van der Meulen et al., 2016). Finally, we tested the different influences of genetics, shared environment and unique environment on social exclusion sensitivity and prosocial behavior in these brain regions. Given that this is a first study examining heritability of fMRI signals in young children, it is important to validate the approach with measures that are more established in genetic designs. Therefore, we also tested the effects of genetics, shared environment and unique environment on total brain volume, a brain measure that has shown consistent heritability in adults (for reviews see Batouli, Trollor, Wen, and Sachdev (2014); Peper et al. (2007) and children (Teeuw et al., 2018). We therefore expected to observe mainly genetic influences on total brain volume in the current sample (see Teeuw et al. (2018), including 9-year-old children).

Methods

Participants

Participants were recruited for the longitudinal twin study of the Leiden Consortium on Individual Development (L-CID). We sent invitations to families with twin children born between 2006 and 2008 in municipalities in the Western region of the Netherlands after obtaining address information from the municipal registries. We included same-sex twin pairs that 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). The initial sample for the L-CID study consisted of 512 participants (256 twin pairs). Since our aim was to study a population sample, participants with a psychiatric disorder were included. In the initial sample, 11 participants were diagnosed with an Axis-I disorder (eight with attention deficit hyperactivity disorder (ADHD) and/or attention deficit disorder (ADD); one with generalized anxiety disorder (GAD), and one with pervasive developmental disorder-not otherwise specified (PDD-NOS). An estimate of IQ was obtained via two subscales (Similarities and Block Design of the Wechsler Intelligence Scale for Children, 3rd version (WISC-III); (Wechsler, 1991). Estimated IQ was within the normal range (range = 72.5 - 137.5).

Twelve participants did not have complete data of the Prosocial Cyberball Game and were therefore excluded from further analyses on prosocial behavior, resulting in a *behavioral sample* of 500 participants (including 244 complete twin pairs in the behavioral twin sample). Of the initial 512 participants, 33 did not perform the Prosocial Cyberball Game in the MRI scanner; 17 due to anxiety, four due to lack of parental consent for the MRI scan, seven due to contra-indications for the MRI scan, and five due to technical errors. Five other participants were excluded from neuroimaging analyses due to anomalous findings, and an additional 191 participants were excluded due to excessive movement (defined as > 3 mm in any volume). This resulted in a *MRI sample* for the neuroimaging analyses of 283 participants, including 89 complete twin pairs in the MRI twin sample (see Table 1 for demographic characteristics of the different samples). A non-response analysis indicated that the participants included in the MRI sample were older ($t(510) = -2.38$, $p = .02$), had a higher estimated IQ ($t(510) = -2.24$, $p = .03$), and were more often female ($X^2 (1) = 9.34$, $p = .004$) than the participants excluded from the MRI sample. There were no significant differences between the monozygotic (MZ) and dizygotic (DZ) twins in the behavioral and MRI twin samples for demographic measures (see Table S1 for demographic information).

Table 1. Demographic characteristics of the samples that were included at various stages of the study.

	Behavioral sample	MRI sample
n	500	283
Complete twinpairs	244	89
Male	48%	42.80%
Left handed	12.60%	12.40%
AXIS-I disorder	11 (2.2%)[1]	7 (2.5%)[2]
Age (SD)	7.94 (.67)	8.01 (.67)
Mean IQ (SD)	103.73 (11.72)	104.57 (12.01)

[1] *9 ADHD and/or ADD; 1 PDD-NOS; 1 GAD.*

[2] *5 ADHD and/or ADD; 1 PDD-NOS; 1 GAD.*

Measures

Prosocial Cyberball Game

To measure behavioral and neural responses to observed social exclusion we used an adapted version of the Prosocial Cyberball Game (PCG; see also Riem et al. (2013); van der Meulen et al. (2017). Participants were instructed to participate in a virtual ball tossing game with three other players, placed at the left (Player 1), the top (Player 2), and the right (Player 3) of the screen. The participant was represented by the figure at the bottom of the screen (see also Figure 1B). Participants were asked to imagine the social setting of the game, such as imaging what the other players and the settings of the game would look like. Previous studies have indicated that imagining playing a game with others is a strong manipulation in gaming research (Konijn et al., 2007), and that exclusion by virtual players leads to reduced feelings of self-esteem (Zadro et al., 2004). We validated this paradigm in earlier studies in children and adults (van der Meulen et al., 2017; van der Meulen et al., 2016).

The PCG was administered in two rounds: a Fair Game and an Unfair Game. In the Fair Game (120 trials), which was administered on a laptop, all four players received the ball an equal number of times (25% of the trials). In the Unfair Game (168 trials), which was administered in the MRI scanner, player 2 was excluded by players 1 and 3 (referred to as the excluding players). The task was programmed in such a way that in case the excluding players were tossing, the participant received the ball on 50% of the trials from the excluding players (they tossed either to the other excluding player (resulting in short intervals of feelings of possible self-exclusion for the participant), or to the participant). In case the excluded player was tossing, the participant received the ball on 33% of the trials (i.e., the excluded participant tossed the ball to the three other players an equal number of times). The Unfair Game was played in two identical blocks, with a short rest period provided between the blocks. Responses during the Unfair Game were recorded through a button box attached to the participant's right leg. Throughout the PCG each trial consisted of one ball toss with a duration of 2000 ms, followed by a jitter with a duration ranging from 1000-2000 ms. When the participant had received the ball, the jitter consisted of the actual response time of the participant.

After completion of the PCG, all participants answered a set of exit-questions to measure their feelings towards the other players in the game. For each of the three players, we asked how much the participants liked that specific player (e.g. "How much did you like player 1?"), indicated by answers on a 6-point Likert scale (1 = not at all, 6 = very much). In addition, we asked participants to whom of the three players they would prefer to donate a sticker (i.e. "If you could donate a sticker to any of the three players, which one would you choose?").

DNA collection

Twin zygosity was determined using information from DNA samples. To this end, buccal cell samples were collected via mouthswab (Whatman Sterile Omni Swab). Samples were collected halfway through the lab visit, to ensure that the children did not eat or drink anything for at least one hour prior to DNA collection. Results of the DNA analyses indicated that 54.9% of the twin pairs in the behavioral twin sample was MZ, whereas 45.1% of the twin pairs in the MRI twin sample was DZ (see Table A.1 for further demographics of the twin samples).

Procedure

Participants received an extensive explanation on the procedure of the MRI scan, as well as a practice session in a mock scanner to further familiarize them with the procedure. All participants performed the Fair Game of the Prosocial Cyberball Game before the scanning session. Participants also performed several behavioral measures as part of the larger L-CID program. 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 performed a social network task (Social Network Aggression Task; Achterberg, van Duijvenvoorde, Bakermans-Kranenburg, & Crone, 2016), and then performed the PCG. After the fMRI tasks, a high resolution structural scan, DTI scans, and a resting state scan were collected. After completing the scanning session, participants answered exit questions about the PCG. After completion of the experimental session, participants received a small goodie bag and parents received financial compensation (€80) for their time, as part of a larger study.

MRI data acquisition

MRI scans were made with a Philips Ingenia MR 3.0 Tesla scanner, using a standard 32-channel whole-head coil. The functional scans were acquired using a T2*-weighted echo-planar imaging (EPI). The first two volumes were discarded to allow for equilibration of T1 saturation effects (TR = 2.2 s; TE = 30 ms; sequential acquisition, 37 slices; voxel size = 2.75 x 2.75 x 2.75 mm; Field of View = 220 x 220 x 112 mm). After the functional runs, 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). Foam inserts were used within the head coil to restrict head movement. Stimuli were projected on a screen, visible via a mirror attached to the head coil.

fMRI data analyses

All data were analyzed with SPM8 (Wellcome Department of Cognitive Neurology, London). Images were corrected for slice timing acquisition and differences in rigid body motion. Functional volumes were spatially normalized to T1 templates. The normalization algorithm used a 12-parameter affine transform together with a nonlinear transformation involving cosine basis functions and resampled the volumes to 3 mm cubic voxels. Templates were based on the MNI305 stereotaxic space (Cocosco et al., 1997). Functional volumes were spatially smoothed with a 6 mm full width at half maximum (FWHM) isotropic Gaussian kernel.

The different events in the PCG-Unfair Game were determined by the tossing of the ball, with the start of each ball toss modeled separately with a zero duration event. To study participants' neural reactions to possible self-exclusion we compared the events of the participant receiving tosses from the excluding players ("Inclusion"; approx. 50% of total tosses from excluding players) to the participant not receiving tosses from these players ("Exclusion; approx. 50% of total tosses from excluding players), and the reversed contrast to examine self-inclusion. To study neural correlates of prosocial behavior we compared the events of the participant compensating for the observed exclusion by tossing the ball to excluded player 2 ("Compensating"; on average 48.1% of total tosses from the participant) to the participant tossing the ball to players 1 and 3 ("Tossing to excluders"; on average

51.9% of total tosses from the participant). We chose these specific contrasts to control for possible confounding factors such as motor preparation or action (i.e. when tossing to one of the other players).

The trial functions were used as covariates in a general linear model; along with a basic set of cosine functions that high-pass filtered the data. The least-squares parameter estimates of height of the best-fitting canonical HRF for each condition were used in pair-wise contrasts. Six motion regressors were included in the first level analysis. The resulting contrast images were computed on a subject-by-subject basis and then submitted to group analyses.

Whole brain analyses

To investigate neural reactions to being excluded from the ball tossing game by the two excluding players, we tested the contrast *Exclusion* > *Inclusion* and the reversed contrast *Inclusion* > *Exclusion*. To investigate neural reactions to prosocial compensating, we tested the contrast *Compensating* > *Tossing to excluders* and the reversed contrast *Tossing to excluders* > *Compensating.*

To test for relations with prosocial behavior, both analyses were followed up with whole brain regression analyses with the behavioral index of prosocial compensating (tossing to player 2 in the Unfair Game – tossing to player 2 in the Fair Game). Condition-related responses were considered significant when they exceeded a FWE-corrected threshold ($p < .05$), or a cluster-corrected threshold of $p < .05$ FWE-corrected, with a primary threshold of $p < .001$ (Woo et al., 2014).

ROI analyses

To select ROIs, we extracted clusters of activation from the whole brain contrasts (Exclusion > Inclusion (and reversed) and Compensating > Tossing to excluders), using the MarsBar toolbox (Brett et al., 2002). To limit the number of regions for further analyses, we used a hypothesis-driven approach to select the final ROIs. Based on prior research, for the *Exclusion* > *Inclusion* contrast, we were primarily interested in the IFG, sgACC, and amygdala (associated with the experience of social exclusion; Cacioppo et al., 2013;

Eisenberger et al., 2007; Vijayakumar et al., 2017), and mPFC (associated with perspective taking and social reasoning; Gunther Moor et al., 2012; Masten et al., 2011). For the reversed *Inclusion* > *Exclusion* contrast, we were interested in the striatum (associated with reward; Delgado, 2007), the bilateral insula, and the ACC (associated with saliency of events; Menon & Uddin, 2010; Seeley et al., 2007). For the contrast *Compensating* > *Tossing to excluders*, we were mainly interested in social brain regions such as the mPFC, precuneus, TPJ and STS (Gunther Moor et al., 2012; Will et al., 2015). After extracting the activation clusters from the whole brain contrasts, we used the MarsBar-AAL (Tzourio-Mazoyer et al., 2002) to mask the a-priori ROIs in the larger activation clusters. Parameter estimates were extracted from the resulting masked ROIs for the conditions "Exclusion" and "Inclusion", and the conditions "Compensating" and "Tossing to excluders". Outliers (z-value <-3.29 or > 3.29) were winsorized (Tabachnick & Fidell, 2013).

Structural MRI data analysis

For the control analysis on total brain volume, we pre-processed T1-scans in FreeSurfer (v5.3.0). Anatomical labeling and tissue classification was performed on the basis of the T1-weighted MR image using the well- validated and well-documented FreeSurfer software (http://surfer.nmr.mgh.harvard.edu/). In short, this software includes tools for non-brain tissue removal, cortical surface reconstruction, subcortical segmentation, cortical parcellation, and estimation of various measures of brain morphometry. Technical details of the automated reconstruction scheme are described elsewhere (Dale, Fischl, & Sereno, 1999; Fischl, Sereno, & Dale, 1999). After pre-processing, each scan was manually checked to assess quality by three trained raters. Each scan was rated as 1 = 'Excellent', 2 = 'Good', 3 = 'Poor', or 4 = 'Failed', based on a set of specific criteria (e.g., affection by movement, missing brain areas in reconstruction, inclusion of dura or skull in reconstruction). After quality assessment, 53 scans (11.2%) were rated as 'Failed' and therefore excluded from further analyses. To check reliability of the three raters, 40 scans were checked by all three raters, resulting in an intra-class correlation of .60.

Total brain volume measures were included for complete twin pairs who also had fMRI data of sufficient quality (n = 166 participants). This sample included 43 MZ twin pairs

(44.2% male) and 40 DZ twin pairs (37.5% male). For these participants, we used automatic subcortical segmentation (Fischl et al., 2002) to extract measures of total gray matter volume ("TotalGray" label, sum of left and right cortical volume, subcortical gray matter volume and cerebellum gray matter) for each participant.

Genetic modeling

To investigate genetic and environmental influences on differences in prosocial behavior, neural reactions to social exclusion and prosocial behavior, and total brain volume, we first computed within-twin pair Pearson correlations for each outcome variable, separately for MZ and DZ twins. A higher correlation for MZ twins would indicate influence of genetic factors, whereas a DZ correlation higher than half the MZ correlation would indicate influence of shared environment. A correlation smaller than 1 indicates an additional effect of unique environment. To further inspect influences of genetic and environmental factors on differences in activity in specific ROIs, prosocial behavior, and total brain volume, we used a structural equation ACE model in the OpenMx package (version 2.7.4; Neale et al., 2016) in R (R version 3.3.2; R Core Team, 2015). With this model we examined the contribution of genetic (A) and shared (C) and unique (E) environmental factors. The E component of the model also included measurement error. For each outcome variable, four different models (ACE, AE (with C set to 0), CE (with A set to 0), and E (with A and E set to 0)) were estimated and a log likelihood was calculated. Each model was then compared to a more parsimonious model (e.g. ACE to AE) by subtracting the log likelihoods, resulting in an estimate of the Log-Likelihood Ratio Test (LRT). Given that the LRT follows the $\chi 2$-distribution, an LRT<3.84 would indicate that the more parsimonious model is a better fit to the data. The Akaike Information Criterion (AIC; Akaike, 1974) was used to determine the best model for equally parsimonious non-nested models (i.e. AE and CE), with better model fit being indicated by a lower AIC.

Since we used a total of nine ACE models to investigate heritability of neural reactions, we were concerned about multiple comparisons and resulting false discovery rate. To counteract this, we performed a supplementary analysis across all ROIs to compute an average heritability estimate of neural activity. For this purpose, we used Falconer's equations (Falconer & Mackay, 1996), with heritability defined as $h^2 = 2 \times (r_{MZ} - r_{DZ})$ and shared environment defined as $c^2 = 2 \times r_{DZ} - r_{MZ}$. We used the within-twin correlations for the MZ and DZ twin pairs for each ROI (also see Table 4). Next, we transformed the within-twin correlations coefficients to Fisher z-values, to stabilize variance. Then we computed an average Fisher z-value for all ROIs for the MZ and DZ twin pairs separately (by adding the Fisher z-values for all ROIs, and dividing that value by the total number of ROIs). Finally, we transformed the Fisher z-value back to correlation coefficients, and we used the resulting correlation coefficients for MZ and DZ twins as variables in the Falconer's equations.

Results

Behavioral results

Prosocial Behavior

The main outcome measure of the PCG is prosocial compensating behavior to excluded Player 2. Since the participants already showed a preference for Player 2 in the Fair Game (see Figure 1A), we defined our outcome measure as the difference in percentage of tosses to Player 2 in the Fair Game and the Unfair Game, to control for behavior in the Fair Game. The percentage of tosses to Player 2 was calculated by dividing the number of tosses to Player 2 by the total number of tosses to all players ((van der Meulen et al., 2016). Using a paired sample t-test in the behavioral sample ($n = 500$), we found that the percentage of tosses from the participant to player 2 was significantly higher when this player was excluded (Unfair Game: $M = 48.44$, $SD = 13.47$, range 5.45-100), compared to the Fair Game ($M = 39.42$, $SD = 10.01$, range 13.33-80.0; $t(499) = -14.09$, $p < .001$, $d = .75$), indicating prosocial compensating behavior. This difference between the Fair Game and the Unfair Game was also significant in the MRI sample ($n = 283$; $t(278) = -10.27$, $p < .001$, $d = .78$), and these results were not affected by age, sex or IQ when these factors were included as covariates in the analyses. Next, we computed the difference score between the Fair Game and Unfair Game. The resulting prosocial compensating score was used as index of prosocial behavior in further analyses. Correlations between percentage of tosses from the participant to the other three players in both the Fair and Unfair Game can be found in Table S2 in the Supplementary Materials.

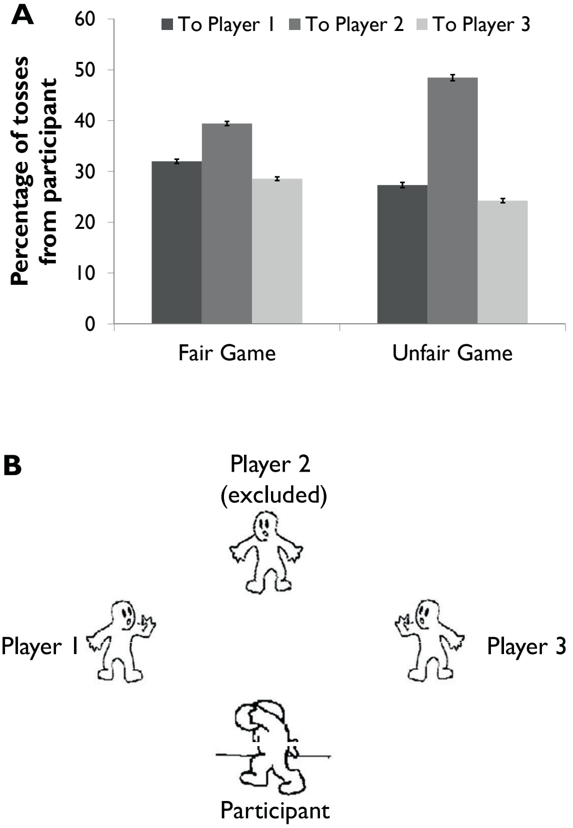


Figure 1. (A) *Percentage of tosses from the participant to the other three players during the Prosocial Cyberball Game.* ***(B)*** *Screenshot of play situation and players in Prosocial Cyberball Game.*

As a validity check for the PCG, we inspected answers on the exit questions. We found that children significantly liked player 2 (M = 5.03, SD = 1.12) more than player 1 (M = 4.29, SD = 1.34; $t(496) = -9.23$, $p < .001$) and player 3 (M = 4.33, SD = 1.46; $t(494) = 8.57$, $p < .001$). There was no significant difference in likeability of player 1 and 3 ($t(494) = .1$, $p = .62$). Next, we computed correlation coefficients for the relationship between prosocial behavior and feelings towards excluded player 2. As expected, prosocial compensating behavior was positively correlated to likeability of player 2 ($r = .14$, $p < .005$), and negatively to likeability of the two excluding players ($r = -.14$, $p < .005$ for player 1 and $r = -.11$, $p < .05$ for player 3), indicating that children who liked player 2 most also showed more prosocial compensating behavior. We also found that the majority of the children chose to donate the sticker to player 2 (60.6%), compared to player 1 (18.9%) and player 3 (20.5%). In addition, we found that children who donated the sticker to player 2 showed more prosocial behavior (M = 11.94, SD = 12.79) than the children who donated the sticker to player 1 (M = 6.38, SD = 15.72) or player 3 (M = 3.37, SD = 13.98; $F(2, 494) = 17.38$, $p < .001$).

Heritability of prosocial behavior

To estimate contributions of genetics, shared environment and unique environment to differences in prosocial behavior after observed exclusion in the behavioral twin sample ($n = 244$ twin pairs, 46.3% MZ) we first computed within-twin correlations for tosses from the participant to player 2 in the Fair and Unfair Game separately. We found no significant associations for MZ or DZ twins (Fair Game $r_{MZ} = .08$ and $r_{DZ} = .18$; Unfair Game $r_{MZ} = -.13$ and $r_{DZ} = -.03$; all p's > .05), indicating no influence of genetics nor shared environment. When performing the same analysis for the difference scores (compensating in Unfair – Fair Game) there were again no positive correlations observed, if anything, the correlation for MZ was negative ($r_{MZ} = -.22$, $p < .05$; $r_{DZ} = -.02$, $p > .05$). Next, we used ACE models to further investigate heritability of prosocial behavior and found that prosocial behavior was best accounted for by unique environmental factors and/or measurement error, with no apparent influence of genetics or shared environment (see Table S3 for full statistics).

Neural results

3.2.1 Whole brain results

The next question concerned the neural regions that were involved in experiencing social exclusion, inclusion and prosocial compensating behavior in the MRI sample ($n = 283$). Since there was a significant difference in age, sex, and IQ between the children included in the MRI sample and the children excluded from the MRI sample, we added the variables age, sex, and IQ as covariates in all whole brain regressions. We first examined neural activity for the experience of possible self-exclusion by conducting a whole brain analysis on the contrast “Exclusion > Inclusion”, defined as not receiving the ball from players 1 and 3 (“Exclusion”) > receiving the ball from players 1 and 3 (“Inclusion”). This contrast resulted in five clusters, including a large cluster spanning the frontal to the occipital cortex, and clusters including mPFC/IFG, and sgACC (see Figure 2A and Table 2 for an overview of all clusters).

To examine neural activity for the experience of self-inclusion, we performed a whole brain analysis on the reversed contrast “Inclusion > Exclusion”. This resulted in eight clusters, including one large cluster spanning the ACC, supplementary motor area, bilateral insula, and bilateral putamen (see Figure 2B and Table 2 for an overview of all clusters). We then examined neural activity for prosocial compensating behavior by performing a whole brain analyses on the contrast “Compensating > Tossing to excluders”, defined as the participant tossing the ball to the excluded players (“Compensating”) versus the participant tossing the ball to the other two players (“Tossing to excluders”). This contrast resulted in one cluster in the posterior cingulate cortex (PCC)/ precuneus (see Figure 2C and Table 3 for an overview of all clusters). The reversed contrast “Tossing to excluders > Compensating” resulted in two clusters, including a large cluster in the visual cortex (see Table 3 for an overview of all clusters).

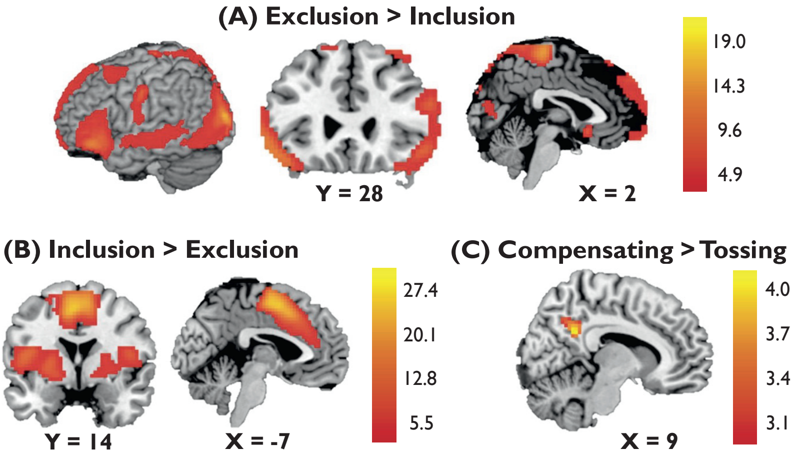


Figure 2. *Whole brain contrasts for* ***(A)*** *Exclusion > Inclusion and* ***(B)*** *Inclusion > Exclusion. Results are reported FWE corrected* $p < .05$*, clusters are only reported in case* $k > 10$*.* ***(C)*** *Whole brain contrast for Compensating > Tossing to excluders, results are reported at a cluster-corrected threshold of* $p < .05$ *FWE-corrected, with a primary threshold of* $p < .001$*.*

Table 2. Whole brain table for the social exclusion contrasts: *Exclusion* > *Inclusion* and *Inclusion* > *Exclusion*. All results are reported FWE corrected $p < .05$, clusters are only reported in case $k > 10$.

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
Exclusion > Inclusion					
L Middle Occipital Gyrus	6521	18.92	-15	-94	-2
		16.64	-21	-94	10
R Superior Occipital Gyrus		16.46	24	-91	13
R Middle Orbital Gyrus	3167	13.3	39	41	-14
R Inferior Frontal Gyrus		12.4	48	41	-14
		10.32	57	35	16
R Insula	19	7.12	39	-16	22
R Hippocampus	37	6.03	21	-22	-11
		4.9	18	-22	-23

Table 2. Continued.

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
L Parahippocampal Gyrus	97	5.4	-21	-19	-20
L Amygdala		5.35	-21	2	-23
L Hippocampus		4.89	-27	-10	-23
L Caudate	15	5.09	-18	5	25
		4.92	-18	-7	28
L Fusiform Gyrus	14	5.03	-33	-34	-23
		4.86	-36	-25	-26
Inclusion > Exclusion					
L Precentral Gyrus	6592	27.36	-39	-25	58
L SMA		22.59	-6	-7	55
L SMA		20.5	-6	11	46
R Cerebellum	36	9.48	21	-55	-20
R Postcentral Gyrus	141	8.87	51	-25	49
R Precentral Gyrus	47	8.58	36	-13	61
L Middle Frontal Gyrus	206	6.89	-39	38	31
R Middle Frontal Gyrus	33	5.88	33	44	31
R Supramarginal Gyrus	16	5.85	54	-22	25
L Precuneus	28	5.46	-15	-64	31

Whole brain regression with prosocial behavior

To test the relation between prosocial behavior and brain activation during prosocial behavior, we conducted a whole brain regression analysis on the contrast “Compensating > Tossing” with the prosocial compensating score as a regressor. The analysis showed that more activity in the left and right insula when tossing to the excluded player was associated with less prosocial compensating behavior (see also Figure 3). The reversed contrast (a positive relationship between prosocial behavior and the contrast “Compensating > Tossing”) did not result in significant activity. For completeness, we also tested the relation between brain activation during possible self-exclusion versus self-inclusion and subsequent prosocial behavior, using a whole brain regression analysis on the contrasts "Exclusion > Inclusion" and "Inclusion > Exclusion". No significant activity was observed in either of the two contrasts.

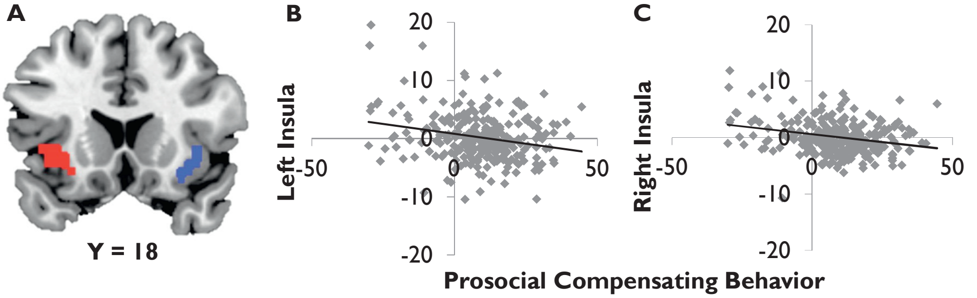


Figure 3. (A) *Activated clusters in whole brain regression with prosocial compensating behavior, with left (red) and right insula (blue).* ***(B)*** *Visualization of regression analysis of prosocial compensating behavior with activity in left insula.* ***(C)*** *Visualization of regression analysis of prosocial compensating behavior with activity in right insula.*

Table 3. Whole brain table for the prosocial contrasts: *Compensating > Tossing to excluders*, *Tossing to excluders > Compensating*, and the whole brain regression for *Compensating > Tossing to excluders*. All results are reported at a cluster-corrected threshold of p < .05 FWE-corrected, with a primary threshold of p < .001.

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
Compensating > Tossing to excluders					
R Posterior Cingulate Cortex	143	3.97	6	-52	31
L Posterior Cingulate Cortex		3.76	-9	-52	28
R Precuneus		3.3	6	-61	40

Table 3. Continued.

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
Tossing to excluders > Compensating					
R Calcarine Gyrus	1121	18.23	12	-85	-2
L Lingual Gyrus		16.44	-9	-85	-5
R Middle Occipital Gyrus		8.13	24	-94	4
L Precentral Gyrus	175	5.5	-30	-10	61
Whole Brain regression: Negative relationship prosocial behavior with contrast Compensating > Tossing to Excluders					
R Insula	266	4.59	42	20	-2
		4.54	39	11	-11
		4.16	36	-4	14
L Insula	160	4.25	-36	20	-5
L Inferior Frontal Gyrus		3.99	-36	38	1
		3.82	-45	17	1

Heritability of brain activity for social exclusion/inclusion and prosocial behavior

To test the contributions of genetics, shared environment, and unique environment to differences in brain activity for the experience of possible self-exclusion and inclusion in the MRI twin sample (*n* =89 twin pairs, 40.4% MZ), we performed follow up analyses on the five ROIs from the contrast “Exclusion > Inclusion” and four ROIs from the contrast “Inclusion > Exclusion”. To test for heritability in neural correlates of prosocial behavior, we selected the cluster in the PCC from the contrast “Compensating > Tossing” (see Figure 2C), as well as the activated clusters from the whole brain regression (left and right insula, see Figure 3A). Since parameter estimates in bilateral ROIs (i.e. IFG and insula) were highly correlated (all *r* > .73), results were collapsed across left and right hemispheres. This resulted in four ROIs for the contrast “Exclusion > Inclusion” (sgACC, smPFC, bilateral IFG, and amygdala), three ROIs for the contrast “Inclusion > Exclusion” (ACC, bilateral insula, and striatum; see Figure 4 for an overview), one ROI for the contrast “Compensating > Tossing” (PCC), and one ROI for the whole brain regression on the contrast “Compensating > Tossing” (bilateral insula).

ACE modelling indicated that differences in activity (in the contrast Exclusion > Inclusion) were partly explained by genetics. Specifically, 33% of variance in smPFC (95% CI: 0-53%) was explained by genetics, whereas in IFG 29% of variance (95% CI: 0-54%) was explained by genetics and 5% (95% CI: 0-45%) was explained by shared environment. All residual variance was best accounted for by the E component (unique environment and measurement error, also see Table 4). Model statistics indicated that an AE model was best fitting for neural activity in these two ROIs (see Table A.3 for full model statistics). Activity in other ROIs from the Exclusion > Inclusion contrast showed minimal to small influences of genetics (amygdala: 2%; 95% CI: 0-27%) and shared environment (sgACC: 10%; 95 CI: 0-30%, also see Table 4).

For the contrast Inclusion > Exclusion, we found small to moderate influences of shared environment for striatum (7%; 95% CI: 0-27%) and bilateral insula (19%; 95% CI: 0-38%). Finally, we found minimal influence of genetics on PCC activity (2%; 95% CI: 0-27%) in the contrast Compensating > Tossing. For these seven ROIs, differences in activity were best accounted for by an E model (see Table A.3). In our supplementary analysis across all ROIs, the results of Falconer’s equations show negligible estimates for both genetic influence ($h^2 = .01$) and shared environmental influence ($c^2 = .08$). Together these findings indicate an overall large contribution of unique environmental influence and measurement error on differences in activity in the selected ROIs.

Control analysis for heritability

We conducted an additional analysis on total brain volume, a structural brain measure, to test genetic contributions. In this analysis we used the residuals of total brain volume, accounted for age, sex, and IQ. We found a high within-twin correlation for MZ twins ($r_{MZ} = .87$, $p < .001$) and a moderate within-twin correlation for DZ twins ($r_{DZ} = .57$, $p < .001$). These within-twin correlations were used to compute Falconer’s estimates of heritability, and we found a strong contribution of genetics ($h^2 = .59$) and a moderate contribution of shared environmental influence ($c^2 = .28$). The more sophisticated ACE modelling also showed a strong genetic component (60%, 95% CI: 26-90%) as well as a contribution of shared environment (26%, 95% CI: 0-58%). Model statistics showed that differences in total brain volume were best accounted for by an AE model (see table S3).

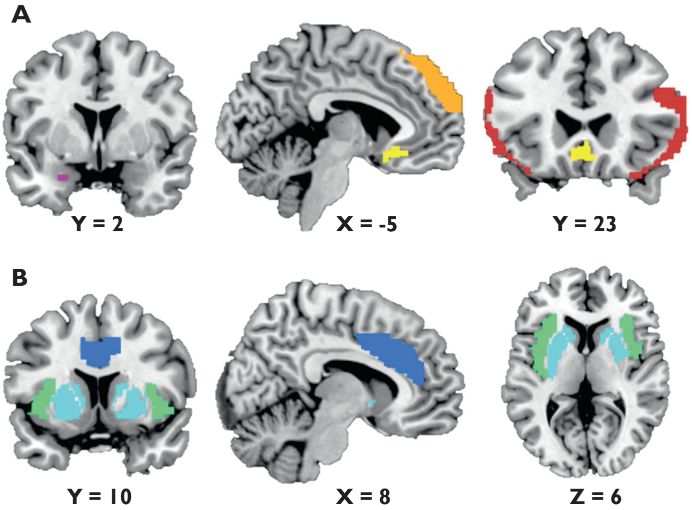


Figure 4. ***(A)*** *Overview of ROIs in the contrast Exclusion > Inclusion: amygdala (dark pink), smPFC (orange), sgACC (yellow), and bilateral IFG (red).* ***(B)*** *Overview of ROIs in the contrast Inclusion > Exclusion: ACC (blue), bilateral insula (green), and striatum (cyan).*

Table 4. Within-twin correlations for MZ and DZ twins and full ACE model estimates with 95% confidence intervals for prosocial behavior and the ROIs associated with the possible experience of self-exclusion and prosocial behavior.

Outcome variable	r_{MZ}	r_{DZ}		A^2	C^2	E^2
Behavior PCG	**-.22***	0	*95% CI*	*† - 0.05*	*† - 0.05*	*0.95 - †*
			ACE	0	0	1
Exclusion > Inclusion						
left amygdala	0.11	-0.14	*95% CI*	*† - 0.27*	*† - 0.19*	*0.73 - †*
			ACE	0.02	0	0.97
IFG	**.37***	0.19	*95% CI*	*0 - 0.54*	*† - 0.45*	*0.46 - 0.89*
			ACE	0.29	0.05	0.66

Table 4. Continued.

Outcome variable	r_{MZ}	r_{DZ}		A²	C²	E²
smPFC	**.36***	0.12	*95% CI*	*0 - 0.53*	*† - 0.40*	*0.46 - 0.91*
			ACE	0.33	0	0.67
sgACC	0.02	0.26	*95% CI*	*† - 0.29*	*† - 0.30*	*0.70 - †*
			ACE	0	0.1	0.9
Inclusion > Exclusion						
ACC	-0.08	0.07	*95% CI*	*† - 0.21*	*† - 0.20*	*0.79 - †*
			ACE	0	0	1
Striatum	-0.08	0.2	*95% CI*	*† - 0.28*	*† - 0.27*	*0.73 - †*
			ACE	0	0.07	0.93
Bilateral insula	0.03	**.31***	*95% CI*	*† - 0.38*	*0 - 0.38*	*0.62 - 1*
			ACE	0	0.19	0.81
Compensating > Tossing						
PCC	0.09	-0.13	*95% CI*	*† - 0.27*	*†- 0.19*	*0.73 - †*
			ACE	0.02	0	0.98
Whole brain regression						
Bilateral Insula	-0.13	-0.06	*95% CI*	*† - 0.18*	*†-0.15*	*0.82 - †*
			ACE	0	0	1
Control analysis						
Total brain volume	**.87***	**.57***	*95% CI*	*0.26 - 0.90*	*0.00 - 0.58*	*0.09 - 0.23*
			ACE	0.6	0.26	0.14

r_{MZ} = within-twin correlation for monozygotic twins, r_{DZ} = within-twin correlation for dizygotic twins. Significant correlations and models are indicated by an asterisk (*) and bold font. † Due to relatively weak correlations, in combination with the sample size, the estimated likelihood function was too flat to accurately estimate 95% confidence interval bounds.

Discussion

The goal of this study was to examine genetic and environmental (shared and unique) influences on experiencing possible self-exclusion and inclusion, and subsequent prosocial compensating behavior when observing exclusion, in 7-9-year-old children. We found that children show prosocial compensating behavior after observing social exclusion by others, which fits well with prior studies in children and adults (Tousignant et al., 2017; van der Meulen et al., 2017; van der Meulen et al., 2016; Vrijhof et al., 2016). Behavioral correlation analyses further showed that participants who demonstrated more prosocial compensating behavior, afterwards liked the excluded player more, and were also more inclined to donate a sticker to the excluded player. These findings suggest that compensation behavior was not solely provoked by inequity aversion or a preference to toss forward (Fehr et al., 2008). The behavior shown during the Prosocial Cyberball Game could also be motivated by a willingness to punish the bullies (i.e. tossing less often to the other two players). However, it seems likely that this motivation would result in overall significantly fewer tosses to the bullies, whereas our participants mainly compensated for exclusion but did not show over-inclusion of the excluded player. Taken together, the overall tendency to toss more balls to the excluded player seems to indicate prosocial and helping motives from the participant.

An important question that we aimed to address was whether prosocial compensation behavior was related to genetic, shared environment, or unique environmental factors. Analyses of heritability revealed only unique environmental and/or measurement error influences on prosocial behavioral differences. Interestingly, previous research showed that parent-reported and self-reported prosocial behavior in children and adolescents was best accounted for by a combination of genetic and unique environmental influences (Gregory et al., 2009; Knafo-Noam et al., 2015; Knafo & Plomin, 2006). Possibly, questionnaires capture more trait-like prosocial behavior, which can partly be accounted for by genetic influences, whereas the Prosocial Cyberball Game elicits more state-like prosocial behavior that is specifically influenced by unique environment. Future research is necessary to examine the genetic contributions of different forms of prosocial behavior, such as based on self-report and in experimental settings.

The neural hypotheses were tested in two steps, first for the experiences of self-exclusion and self-inclusion, and second for subsequent prosocial compensating behavior after observing social exclusion of another player. First, experience of possible self-exclusion was associated with activity in an affective salience network including IFG, amygdala, and sgACC. Whereas the association between social exclusion and activity in IFG and sgACC has consistently been reported in both adolescents and adults (Cacioppo et al., 2013; Rotge et al., 2015; Vijayakumar et al., 2017), amygdala activity is less often associated with experience of social exclusion (Eisenberger et al., 2007). Together, these findings indicate that experience of possible social exclusion might be a meaningful event for children. Additionally, experience of inclusion was associated with activity in bilateral insula, ACC, and striatum. These regions have previously been associated with signaling appraisal of upcoming events (Shenhav et al., 2016) and reward processing (Bhanji & Delgado, 2014; Delgado, 2007), indicating the importance of being included in middle childhood. In interpreting these findings it should be noted that the experience of social exclusion and inclusion in the Prosocial Cyberball Game differs from experiencing social exclusion in the traditional Cyberball Game, as participants in our study were only excluded for short periods of time (3-5 trials) before being included again. Therefore, it is possible that the short intervals of social exclusion were only processed implicitly by the participants. In addition, the event of not receiving the ball from the other two players might have resulted in expectancy violation or disengagement from the task in the participant, thereby decreasing the experience of social exclusion. However, the alternating pattern of acceptance and rejection in the Prosocial Cyberball Game is comparable to other paradigms that have measured responses to social rejection by providing alternating positive and negative feedback to participants (Achterberg et al., 2016; Gunther Moor, Crone, & van der Molen, 2010; Silk et al., 2014; Somerville, Heatherton, & Kelley, 2006).

We were also interested in testing how children act upon observing social exclusion. Whereas earlier studies in adults showed that more prosocial behavior was related to increased activity in mPFC, we found that in children prosocial compensating was associated with increased activity in PCC/precuneus. This region has previously been associated with retrieval of social information (Pfeifer et al., 2007) and empathy (Masten et al., 2011), and is seen as part of the default mode network that specializes in mentalizing

(Hyatt, Calhoun, Pearlson, & Assaf, 2015). Interestingly, in previous research in adults more prosocial behavior was related to activity in the TPJ (van der Meulen et al., 2016), which has also been associated with mentalizing and perspective taking (Carter & Huettel, 2013; Hyatt et al., 2015). We also found a negative association between prosocial behavior and activity in the bilateral insula, consistent with other studies towards insula activation during prosocial behavior in adolescence (Güroğlu et al., 2014; Schreuders, Klapwijk, Will, & Güroğlu, 2018). However, previous research in adults showed a positive association between activity in bilateral insula and prosocial compensating towards the excluded player (van der Meulen et al., 2016). Together, these findings suggest that the brain network involved in mentalizing and prosocial behavior continues to develop from childhood to adulthood. Possibly, the PCC holds the function of mentalizing during childhood, while the TPJ holds this function in adulthood. The function of the bilateral insula possibly changes to accommodate this shift in function. Longitudinal studies are necessary to examine this in more detail.

When we investigated heritability of the neural reactions towards social exclusion, we found that across all ROIs differences in activity were best accounted for by unique environmental factors and measurement error. Although heritability of brain function has received little attention in earlier studies, our overall findings fit with a prior study on heritability of the neural correlates of social rejection in middle childhood (Achterberg et al., 2018) that also reported large influences of unique environment and/or measurement error, and smaller influences of genetics (estimated between 10-14%). In the current study, ACE models showed significant genetic contributions in two out of our nine ROIs: We found that differences in activity in IFG and smPFC during the experience of possible self-exclusion were best accounted for by genetic (estimated 29-33%) and unique environmental factors/measurement error. These findings are partly consistent with earlier research showing that there is more similarity in activity during cognitive tasks in adult MZ twins than DZ twins, with estimated influences of genetics ranging from 40-65% (Jansen et al., 2015). It should be noted that earlier studies towards the heritability of brain function mostly studied well-defined and highly reliable processes (e.g. processing of visual stimuli; Polk, Park, Smith, & Park, 2007). Possibly, genetic effects are less pronounced in brain activity for complex social situations (such as social exclusion) that encompass relatively more individual differences, due to a strong interplay of genetics and personal experiences (van Harmelen et al., 2014; van Schie et al., 2017). In the current study, we had no a-priori hypotheses for the selected ROIs and the outcomes of the ACE models were not corrected for multiple comparisons. Our sample size for heritability analyses was also relatively small (*n* = 168), but comparable to earlier studies in infants and children (Achterberg et al., 2018; Achterberg et al., 2018; Bakermans-Kranenburg, van Uzendoorn, Bokhorst, & Schuengel, 2004; van den Heuvel et al., 2013). Therefore, our findings should be regarded as exploratory and as a starting point for future studies towards heritability of neural activity for complex social situations.

Our control analysis on heritability of total brain volume showed that differences in total brain volume were accounted for by genetic (86%) and unique environmental factors. This finding fits well with earlier research on heritability of total brain volume in children, which indicated moderate to high heritability in children (Jansen et al., 2015; Teeuw et al., 2018). Possibly, the divergence in our findings for brain activity and brain structure can be explained by the fact that brain structure is a more stable measure, whereas brain activity for complex social stimuli shows more state-like characteristics. For example, it has been found that neural responses to social exclusion in a Cyberball Game change when participants feel emotionally supported (Onoda et al., 2009), whereas brain structure is less affected by these individual state differences. In addition, functional MRI has relatively lower signal to noise ratio than structural MRI, especially for social processes (Lieberman & Cunningham, 2009) such as social exclusion and prosocial behavior. The larger amount of noise in functional MRI could lead to an overestimation of unique environment and/or measurement error, compared to structural MRI. A direction for future studies would be to focus on disentangling influence of measurement error and unique environment in the E component (for example by using repeated measures to account for intra-subject fluctuations as described by Ge, Holmes, Buckner, Smoller, and Sabuncu (2017)), as this is currently not possible in ACE modelling. Nonetheless, the finding of heritability of total brain volume shows that the current sample size is sufficient to estimate heritability of if within-twin correlations are at least moderately strong.

Strengths and limitations

This study had several strengths. To our knowledge this is the first research conducted on neural correlates of experiencing self-exclusion, self-inclusion, and prosocial behavior, with a large sample size that allowed us to investigate individual differences. Moreover, the twin design allowed us to test for influences of genetics and shared and unique environment on differences in neural activity during social exclusion and prosocial behavior, which has not been investigated before.

Some limitations of this study should also be noted. First, we now focused on the Unfair Game to control for time effects and to allow for a clear comparison of tossing to excluded and excluding players. An adaptation of this design in future studies would provide further insight in prosocial compensating behavior by also examining brain activity during the Fair Game. Additionally, in the current design it is difficult to differentiate between different motives for engaging in prosocial behavior. Although it is an advantage that we now studied reactions to observed social exclusion in a relatively controlled environment, for future studies it would be interesting to also investigate a more diverse battery of prosocial tasks. Second, although our neural findings for the experience of possible self-exclusion are comparable to earlier studies on social rejection, we have no additional information on the participant's experience of being excluded. For future studies, exit questions about how participants felt when they did not receive the ball from excluding players 1 and 3 could lead to more insight in the participant's experience. Third, genetic contributions for fMRI were relatively low and were only observed for one contrast (possible self-exclusion). Therefore, not all brain areas that were selected as ROIs might be equally suitable for investigating influences of genetics and environment. For example, amygdala activity has shown low to moderate test-retest reliability in an earlier study (Sauder, Hajcak, Angstadt, & Phan, 2013; van den Bulk et al., 2013), indicating that this might not be a very stable outcome measure (but see Lumian and McRae (2017)). In addition, the ROIs selected in the current study were based on clusters of whole brain activation, thereby decreasing individual variation in activation of these brain regions. Possibly, this has made the current selection of ROIs less suitable for discovering genetic and environmental influences on differences in brain activity. Final recommendations for future research would be to also include a control task (preferably not aimed at measuring social exclusion) to test whether the current heritability results on neural activation are specific for a social exclusion context, or whether these results are applicable for brain activity in various brain regions. In addition, it would be very interesting to study overlapping effects of genetics and environment on both behavior and brain activation, to find out whether similar heritability mechanisms are driving differences in behavior and brain activation.

Conclusion

The current study builds upon the existing literature by showing that children show prosocial compensating behavior when they observe social exclusion. Further, although we note that certain conclusions are based on reverse inference, our findings suggest that children experience possible social self-exclusion as a negative event (as indicated by activity in IFG, smPFC and amygdala), inclusion as a positive and salient event (as indicated by striatum and ACC-insula activity) and that prosocial compensating behavior is partly driven by mentalizing capacities (as indicated by activity in PCC). Heritability analyses showed that differences in both prosocial behavior and neural activity during possible self-exclusion and prosocial behavior are potentially driven by unique environmental factors, but since measurement error is relatively high in fMRI research due to higher signal-to-noise ratio's, at this point the role of unique environment versus measurement error remains inconclusive. In future research, it will be important to study the neural processes and heritability profiles across multiple stages of development, and to test for heritability estimates of activity in specific brain regions to further investigate sensitive periods in development.

Supplementary Materials

Table S1. Demographic characteristics of the MZ and DZ samples for behavioral and MRI twin analyses. No significant differences in demographic measures between MZ and DZ twins in either sample were found.

	Behavioral twin sample		MRI twin sample	
	MZ	DZ	MZ	DZ
n	268	220	94	84
Male	46.30%	48.20%	40.40%	40.50%
Left handed	12.70%	11.40%	13.80%	10.70%
AXIS-I disorder	3 (1.1%)[1]	7 (3.2%)[2]	0 (0%)	3 (3.6%)[3]
Age (SD)	7.96 (.70)	7.93 (.64)	8.07 (.74)	7.91 (.57)
Mean IQ (SD)	103.92 (12.46)	103.61 (10.84)	105.50 (13.50)	103.72 (11.27)

[1] *3 ADHD and/or ADD*

[2] *6 ADHD and/or ADD; 1 PDD-NOS; 1 GAD*

[3] *2 ADHD and/or ADD; 1 PDD-NOS*

Table S2. Correlations between percentage of tosses from the participant to the three other players in the Fair and Unfair Game of the Prosocial Cyberball Game.

			Fair			Unfair	
		Player 1	Player 2	Player 3	Player 1	Player 2	Player 3
	to Player 1	-					
Fair	to Player 2	-.60***	-				
	to Player 3	-.37***	-.53***	-			
	to Player 1	.22***	-.16***	-0.05	-		
Unfair	to Player 2	-.20***	.28***	-.11*	-.69***	-	
	to Player 3	0.03	-.21***	.21***	-.19***	-.58***	-

Table S3. Full ACE models for prosocial behavior and the ROIs associated with the possible experience of self-exclusion and prosocial behavior.

Outcome variable	model	A^2	C^2	E^2	LTR	AIC
Behavior PCG	ACE	0	0	1		3002.5
	AE	0	-	1	< 0.001	3000.5
	CE	-	0	1	< 0.001	3000.5
	E*	**-**	**-**	**1**	**< 0.001**	**2998.5**
Exclusion > Inclusion						
left amygdala	ACE	0.02	0	0.97		701.65
	AE	0.02	-	0.97	< 0.001	699.65
	CE	-	0	1	0.04	699.68
	E*	-	-	**1**	**< 0.04**	**697.68**
IFG	ACE	0.29	0.05	0.66		324.22
	AE*	**0.34**	**-**	**0.66**	**0.02**	**322.25**
	CE	-	0.29	0.71	0.53	322.76
	E	-	-	1	> 7.65	328.41
smPFC	ACE	0.33	0	0.67		369.45
	AE*	**0.33**	**-**	**0.67**	**< 0.001**	**367.45**
	CE	-	0.25	0.75	1.38	368.83
	E	-	-	1	> 5.68	372.52
sgACC	ACE	0	0.1	0.9		404.36
	AE	0.08	-	0.92	0.53	402.89
	CE	-	0.1	0.9	< 0.001	402.36
	E*	**-**	**-**	**1**	**< 0.93**	**401.3**

Table S3. Continued.

Outcome variable	model	A^2	C^2	E^2	LTR	AIC
Inclusion > Exclusion						
ACC	ACE	0	0	1		426.14
	AE	0	-	1	< 0.001	424.14
	CE	-	0	1	< 0.001	424.14
	E*	**-**	**-**	**1**	**< 0.001**	**422.14**
Striatum	ACE	0	0.07	0.93		475.98
	AE	0.04	-	0.96	0.4	474.38
	CE	-	0.07	0.93	0	473.98
	E*	**-**	**-**	**1**	< 0.47	**472.45**
Bilateral insula	ACE	0	0.19	0.81		465.74
	AE	0.19	-	0.81	1.37	465.12
	CE	-	0.19	0.81	< 0.001	463.74
	E*	**-**	**-**	**1**	**< 3.15**	**464.89**
Compensating > Tossing						
PCC	ACE	0.02	0	0.98		716.74
	AE	0.02	-	0.98	< 0.001	714.74
	CE	-	0	1	0.02	714.76
	E*	**-**	**-**	**1**	**< 0.02**	**712.76**
Whole brain regression						
Bilateral Insula	ACE	0	0	1		531.22
	AE	0	-	1	< 0.001	529.22
	CE	-	0	1	< 0.001	529.22
	E*	**-**	**-**	**1**	**< 0.001**	**527.22**

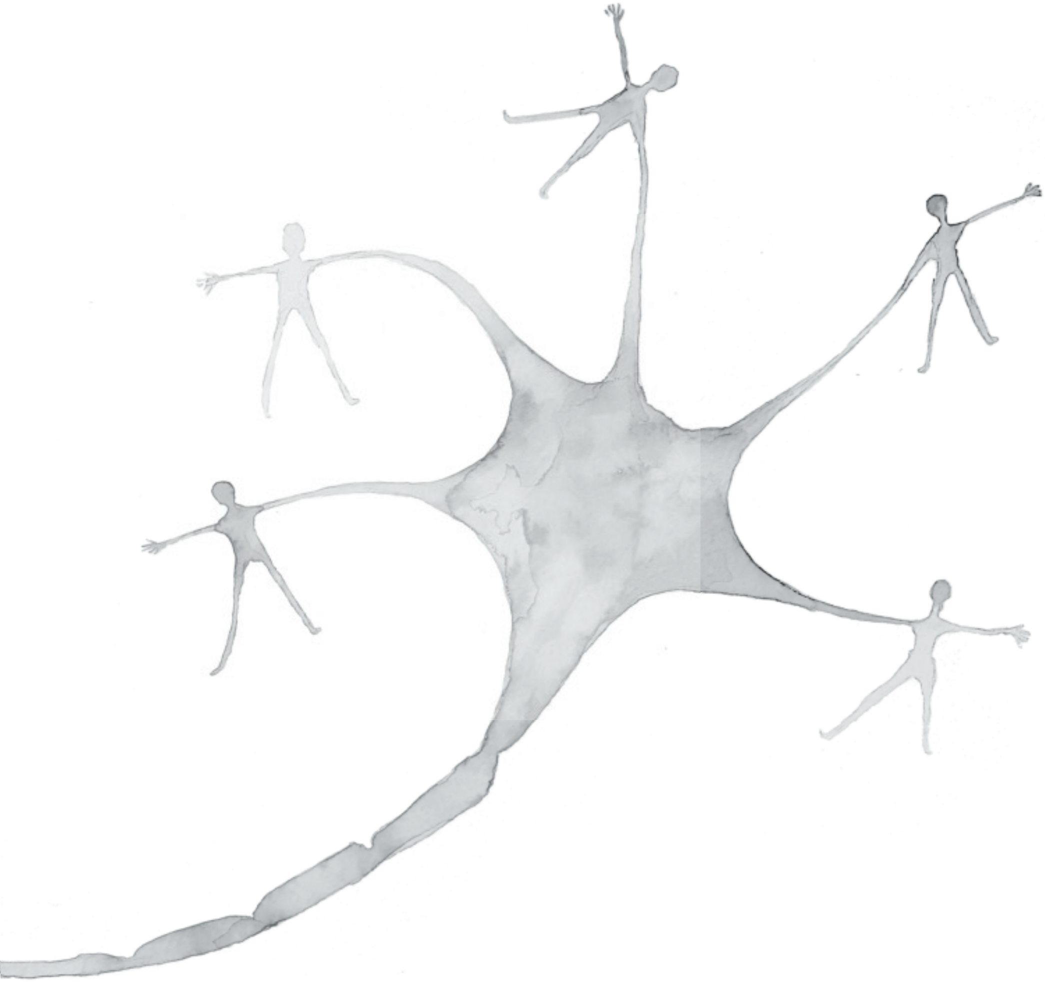
Table S3. Continued.

Outcome variable	model	A^2	C^2	E^2	LTR	AIC
Control analysis						
Total brain volume	ACE	0.6	0.26	0.14	1.31	1387.01
	AE*	**0.86**	**-**	**0.14**	**13.46**	**1386.32**
	CE	-	0.72	0.28	71.74	1398.47
	E	-	-	1	59.61	1456.07

Significantly best fitting models are indicated in bold font, with an asterisk (*).

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.

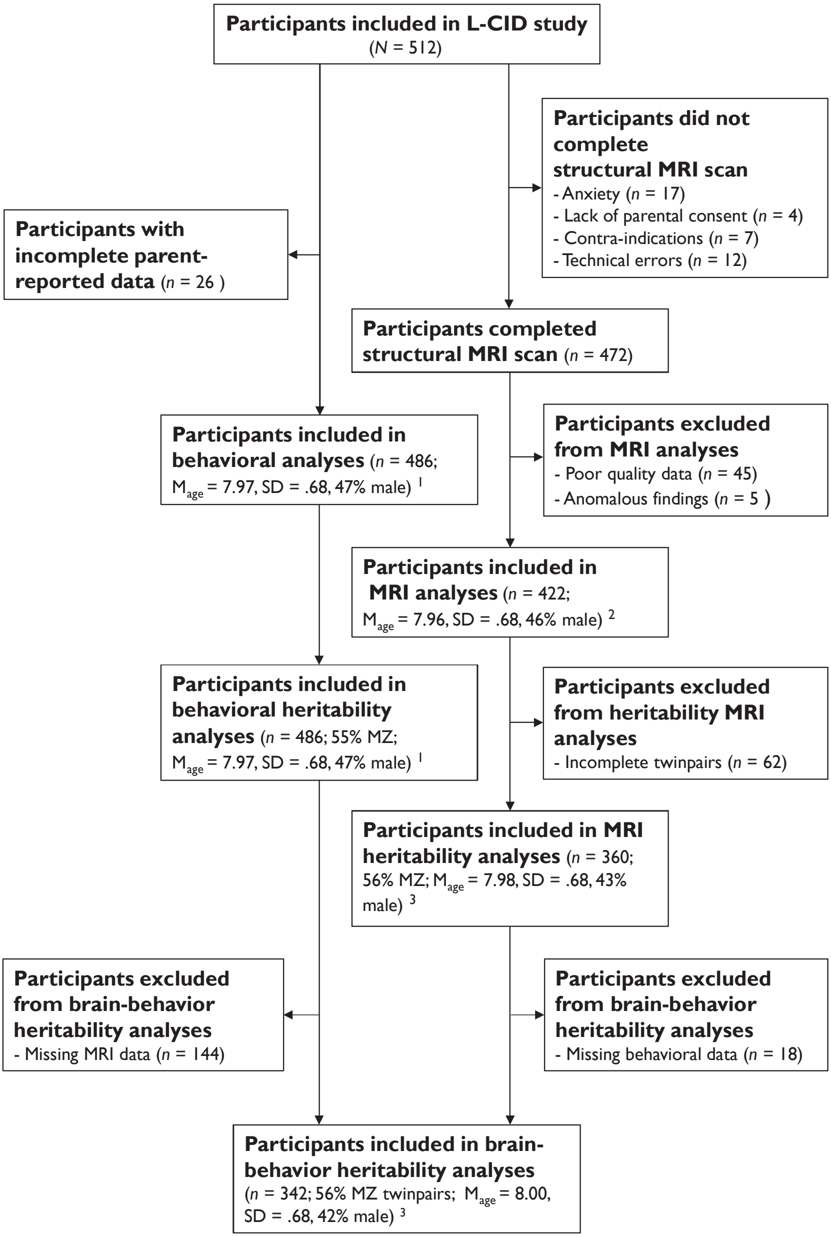


Figure 1. *Flowchart of inclusion of samples (including demographic information) at various stages of the study. MZ = monozygotic twin pairs; [1] Diagnosed Axis-I disorders: ADHD and/or ADD (eight participants), PDD-NOS (one participant), generalized anxiety disorder (GAD; one participant); [2] Diagnosed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

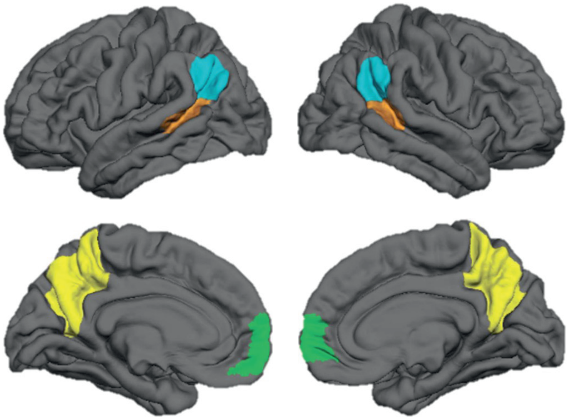


***Figure 2**. Regions of interest in the social brain, including TPJ (blue), pSTS (orange), mPFC (green), and precuneus (yellow). Left side of the panel indicates left hemisphere, right side of the panel indicates right hemisphere.*

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 α = .003 for the 17 associations (α=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.

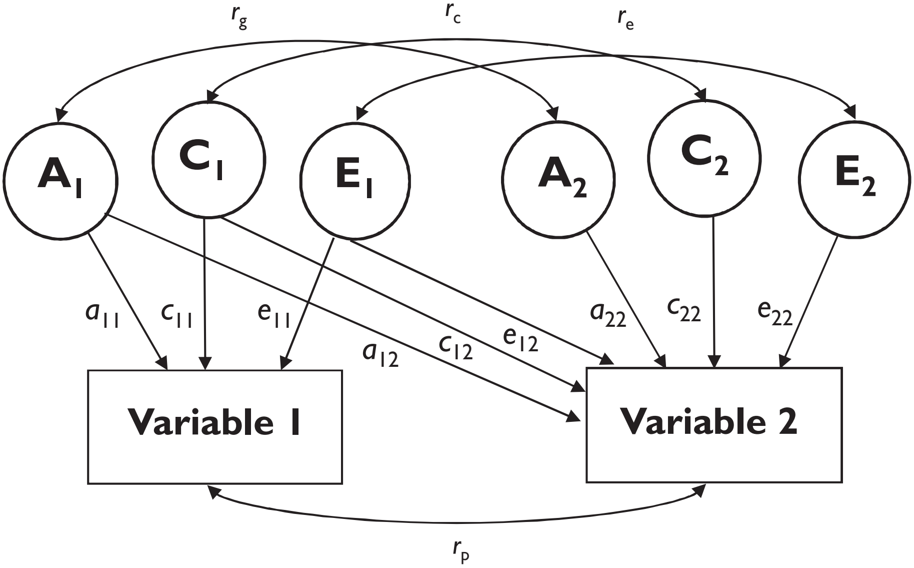


Figure 3. *Bivariate ACE model, visualizing contributions of genetic (A), shared environmental (C) and unique environmental (E) on two variables. Paths* a_{11}-e_{11} *and* a_{22}-e_{22} *indicate contributions of genes and environment on variables 1 and 2, respectively. Paths* a_{12}-e_{12} *indicate 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 ($\beta = .44$, $t(482) = 6.96$, $p < .001$). 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 behavior	ACE	0.45	0	0.55		-329.76
	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

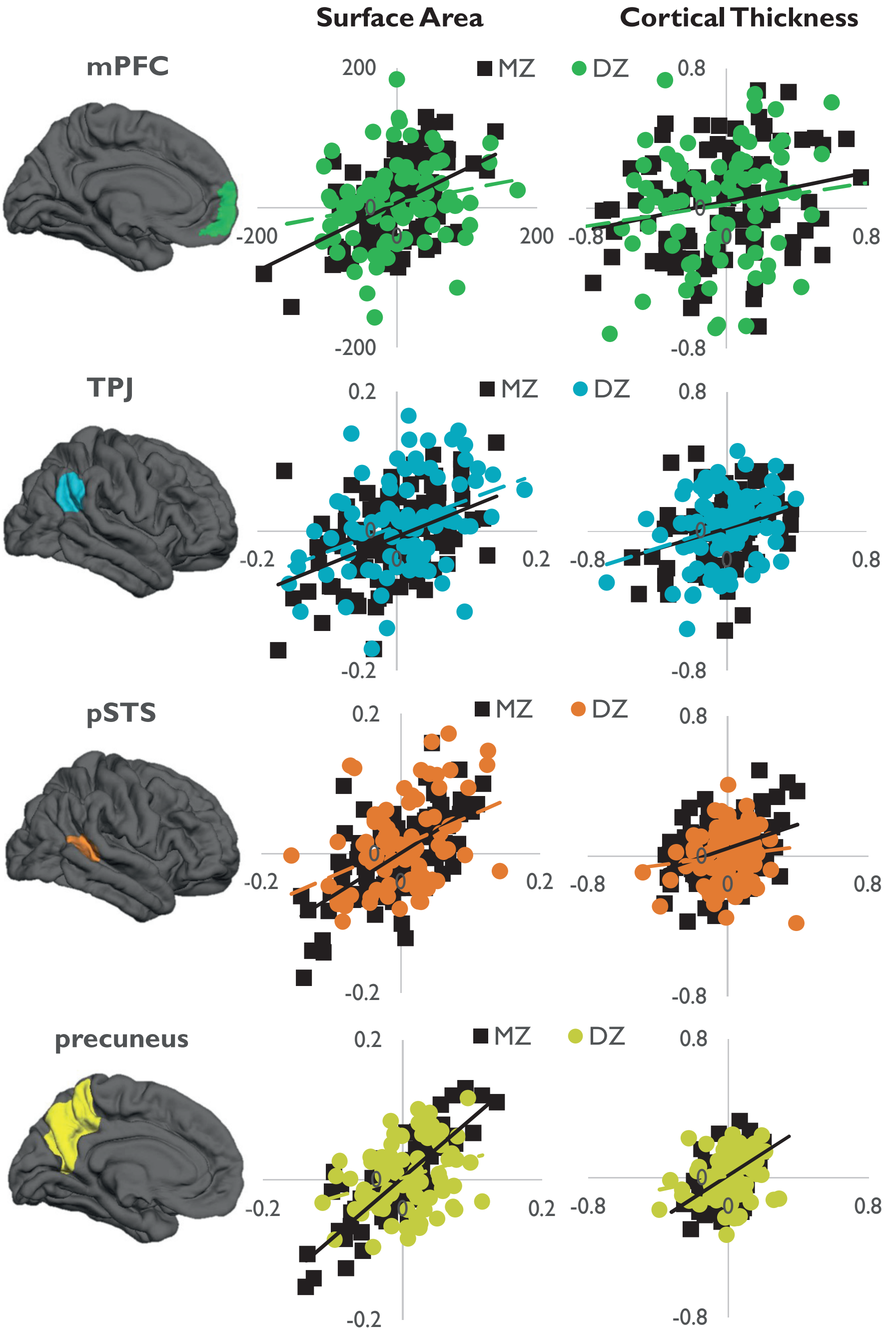
Best fitting models are indicated by an asterisk (*) and bold font.

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

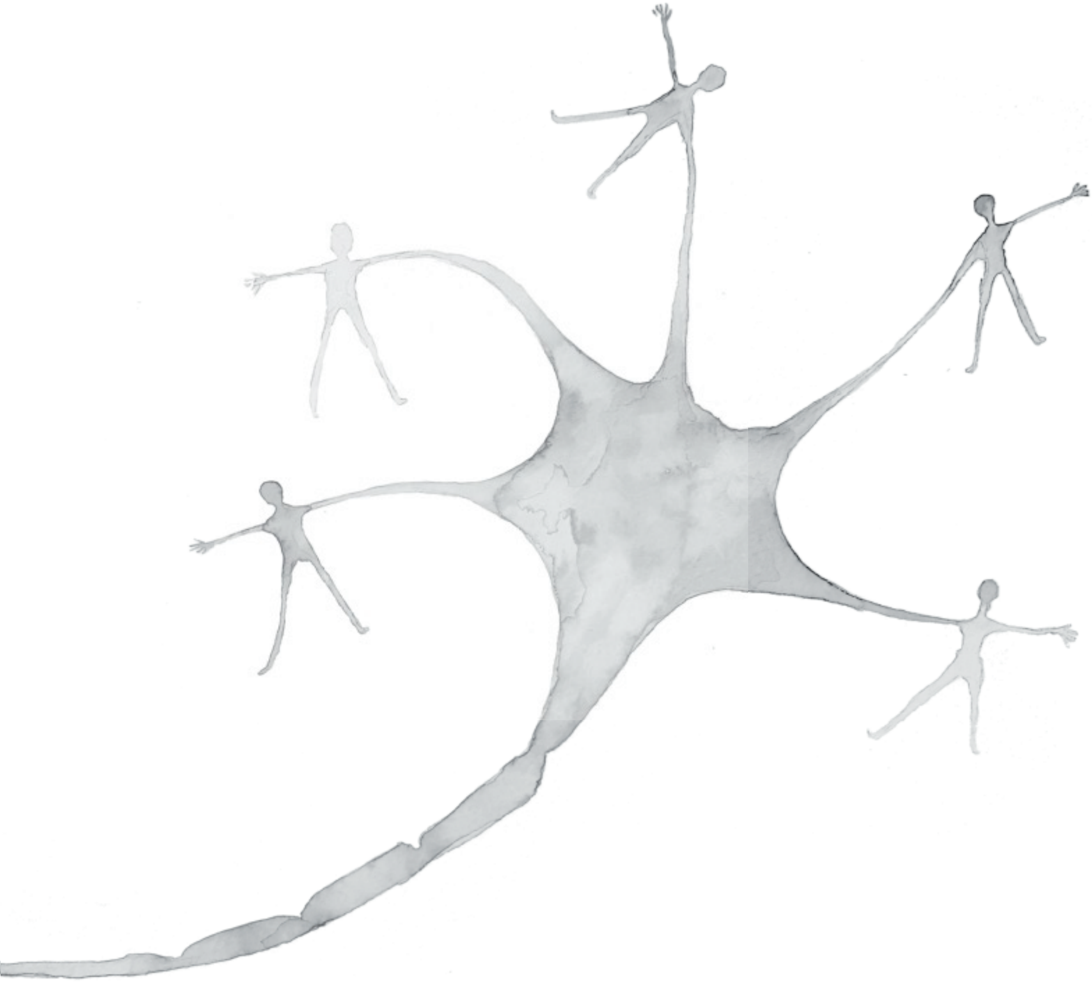
Best fitting models are indicated by an asterisk (*) and bold font.

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
■ MZ
● DZ
200
-200
0
-0.8
0.8
TPJ
0.2
-0.2
pSTS
precuneus

CHAPTER 6

General discussion



Summary

The main goal of this thesis was to investigate the behavioral and neural processes involved in social competence in middle childhood. First, I studied processes that contribute to social competence by looking into prosocial behavior and its associated neural mechanisms in children and young adults (**chapters 2, 3** and **4**). Further, I examined the association between structure of social brain regions and parent-reported prosocial behavior (**chapter 5**). Second, I studied processes that might negatively affect social competence by measuring neural responses to the experience of social exclusion (**chapters 3** and **4**). Finally, I estimated influences of genetics and environment on social competence and associated neural processes (**chapters 4** and **5**). In this final chapter of the thesis I first provide a summary of the main findings of these studies. A visual summary of the brain regions associated with prosocial behavior and social exclusion (see Figure 1). This section is followed by a general discussion, future directions and conclusions.

Social competence in adults

In **chapter 2**, I aimed to disentangle neural underpinnings for observing social exclusion and acting prosocially by employing a paradigm that allows for the investigation of both processes simultaneously. A sample of young adult females (*N* = 23; aged 18-19) played the Prosocial Cyberball Game (PCG; adapted from Riem et al. (2013)) during a MRI session. In this four-player ball tossing game, participants first played a fair block with three computerized players. During the fair situation all players received the ball an equal amount of times. Next participants played an unfair block, during which one of the players was excluded. This provided participants with the opportunity to compensate for the exclusion by tossing more balls to the excluded player and thereby show prosocial compensation behavior.

Behavioral findings indicated that participants tossed more balls to the excluded player compared to the two excluding players, thereby engaging in prosocial compensation behavior. Neuroimaging analyses revealed that tossing the ball to the excluded player was associated with in increased activity in bilateral insula, bilateral temporo-parietal junction

(TPJ) and bilateral nucleus accumbens (NAcc). In addition, participants showed neural activity in bilateral insula when observing exclusion. Together, these findings show that bilateral insula plays a role in both prosocial behavior and observing social exclusion, possibly indicating that both events are interpreted as meaningful events in a social context. Activation in social cognition and reward processing areas (e.g. TPJ and NAcc) was specific for prosocial behavior, thereby highlighting the dual role of other- (i.e. taking another's perspective) and self-oriented (i.e. experiencing a feeling of reward) motivations when engaging in prosocial behavior. With these findings as a starting point, I now turn to the three experimental studies conducted in middle childhood.

A test and replication study of social competence in childhood

I aimed to disentangle self- and other oriented concerns in middle childhood by studying prosocial behavior and concerns about experiencing social exclusion in **chapter 3**. A second aim of this study was to try and overcome some of the concerns raised about replicability of psychological experiments (Open Science, 2015), by using a replication and meta-analysis approach. In this study three middle childhood samples (aged 7-10) performed the PCG during a MRI session. Behavioral findings indicated that across all three samples children engage in prosocial compensation behavior in a situation of observed social exclusion. No significant activation in social brain regions was found in any of the three samples separately, nor in a meta-analysis. These findings demonstrate that children consistently show prosocial behavior towards an excluded player in a virtual ball tossing game, comparable to the findings in **chapter 2**. When investigating the experience of social exclusion, I found significant clusters of activation in the left orbitofrontal cortex (OFC) and occipital lobe in all three samples. During the experience of social inclusion, children showed increased activation in clusters spanning dorsal anterior cingulate cortex (dACC)/medial prefrontal cortex (mPFC), bilateral insula, ventral striatum and pre- and postcentral gyrus in all three samples. A meta-analysis indicated common activation of OFC for social exclusion, and activation of dACC and sections of the ventral striatum for social inclusion across the three samples. In sum, children show consistent neural reactions to the experience of social exclusion and inclusion in regions previously associated with perception management and reward processing, respectively. Underlying neural processes of prosocial behavior were less evident, possibly due to the large variation in prosocial behavior. This highlights the need for larger samples in research towards neural processes associated with prosocial behavior.

Heritability of social competence and brain function

To address the need for larger samples and to understand how genes and environment affect social competence, I studied the heritability of neural processes underlying prosocial behavior and social exclusion in **chapter 4**. In this experimental study a large middle childhood twin sample performed the PCG during a MRI session. Children showed prosocial compensation behavior towards a socially excluded player in the PCG, with neuroimaging findings revealing an increase in activity in the precuneus when participants behaved prosocially. An additional whole-brain regression analysis indicated that participants who demonstrated more prosocial behavior showed less insula activity when tossing to the excluded player. I interpreted these findings to indicate that prosocial compensation behavior in middle childhood is supported by underlying mentalizing processes (i.e. precuneus activity) as well as awareness of social norms (i.e. activity in bilateral insula). Both behavioral and neural findings were influenced by unique environmental factors and/or measurement error, possibly alluding to the strong influence of specific context on prosocial behavior. Regarding the experience of social exclusion, neuroimaging results showed increased activity in clusters spanning superior medial prefrontal cortex (smPFC)/inferior frontal gyrus (IFG) and subgenual ACC during social exclusion, and increased activity in a large cluster encompassing bilateral insula, bilateral putamen and dACC during social inclusion. Behavioral genetic analyses indicated that variance in neural activity in IFG and smPFC (during the experience of exclusion) was driven by a combination of genetic and unique environmental factors. Activity in other regions was best explained by unique environmental factors. In the current study unique environmental factors could not be separated from measurement error. In brief, this study showed that neural processes related to prosocial behavior and social inclusion might be more sensitive to environmental influences, as indicated by the influence of unique environmental factors and/or measurement error. Neural processing of social exclusion showed a small genetic effect in addition to unique environmental factors.

Heritability of prosocial behavior and brain structure

Turning to the association between social competence and brain structure, in **chapter 5** I studied whether similar genetic and environmental influences account for variance in structure of the social brain and prosocial behavior. I extracted measures of surface area and cortical thickness from four a-priori specified regions of interest (i.e. mPFC, TPJ, posterior superior temporal sulcus (pSTS) and precuneus) in the same middle childhood sample as described in **chapter 4**. Prosocial behavior was measured via parent-reported questionnaires. Findings from univariate behavioral genetic modelling showed substantial contributions of genetic factors to surface area of mPFC, pSTS and precuneus, whereas surface area of TPJ was influenced by both genetic and shared environmental factors. In contrast, cortical thickness of the precuneus was also largely driven by genetic factors, while cortical thickness of mPFC, pSTS and TPJ was influenced by both genetic and shared environmental factors. Finally, prosocial behavior and empathy were substantially influenced by genetic factors in addition to unique environmental factors. Bivariate genetic modelling showed a small overlap between genetic and unique environmental factors for cortical thickness of precuneus and empathy, indicating that some genetic and unique environmental factors account for lower cortical thickness of precuneus and higher levels of empathy. I also found a moderate overlap between genetic and unique environmental factors for prosocial behavior and empathy. Together these findings showed that both structure of the social brain and prosocial behavior are largely heritable, but that only a small amount of genetic factors overlap for brain structure and behavior.

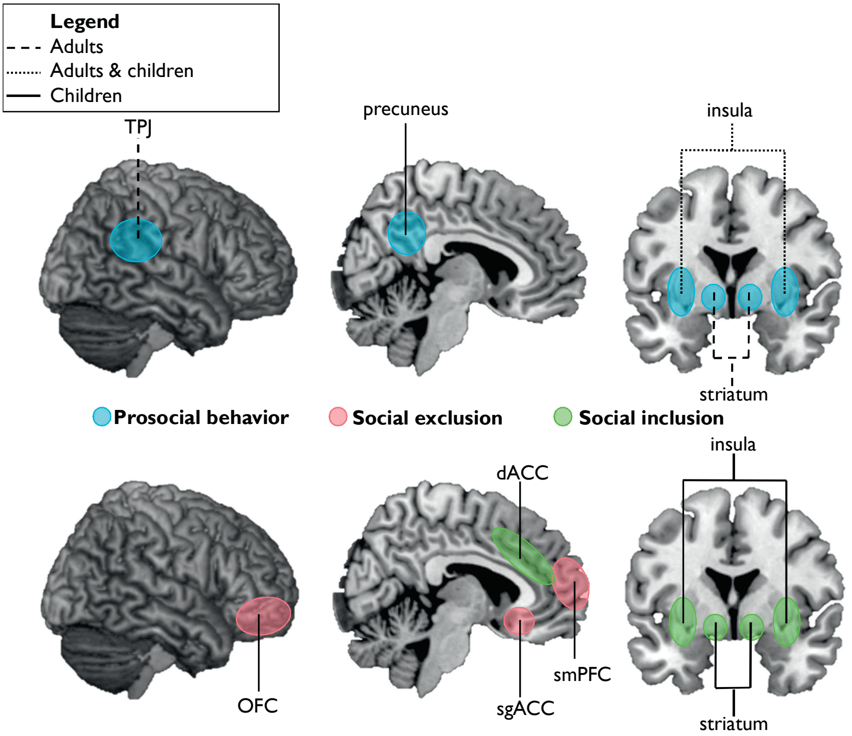


Figure 1. *Visual summary of findings with regard to functioning and structure of the social brain. Areas highlighted in blue were associated with prosocial behavior; temporo-parietal junction (TPJ), precuneus, insula and striatum. Areas highlighted in pink were associated with social exclusion; orbitofrontal cortex (OFC), subgenual anterior cingulate cortex (sgACC), superior medial prefrontal cortex (smPFC). Areas highlighted in green were associated with social inclusion; dorsal anterior cingulate cortex (dACC), insula, and striatum.*

General discussion

Neural signature of prosocial behavior

The studies that are part of this thesis can be summarized in a few main findings. First, I show that observing social exclusion results in prosocial compensation behavior in childhood (**chapters 3 and 4**) and adulthood (**chapter 2**). In other words, I found that participants adapted their behavior towards an unfamiliar player when that player was excluded. Brain regions that were activated during prosocial compensation behavior (e.g. TPJ in adults, precuneus in children) have previously been associated with the socio-cognitive processes of social competence (Blakemore, 2008), suggesting the presence of underlying mentalizing processes during the compensation of observed exclusion. Interestingly, neural structure of the precuneus was also associated with empathy (seen as a prerequisite for prosocial behavior; Eisenberg et al. (2015)) in middle childhood (as shown in **chapter 5**). More specifically, lower levels of cortical thickness of the precuneus was related to more empathic behavior. As maturation of the brain is characterized by cortical thinning (Mills et al., 2016; Wierenga et al., 2014), it is possible that higher levels of empathy reflect increased maturation of the social brain regions. However, the exact direction of this brain-behavior association needs to be determined in follow-up longitudinal studies.

In addition to neural regions involved in socio-cognitive processing, prosocial compensation behavior was also associated with brain regions associated with socio-affective processing. That is to say, in children I found that individuals who showed less prosocial behavior showed increased insula activity during prosocial behavior. Possibly, this neural activation signals an awareness of crossing social norms, as the insula has been shown to be involved in acting against social norms (Güroğlu et al., 2011). This might be particularly salient in middle childhood as this is the developmental stage of social norm internalization (Smith, Blake, & Harris, 2013). Furthermore, adults showed increased activity in the NAcc, a region associated with reward processing, during prosocial behavior. This finding fits with the hypothesis that prosocial behavior might be driven by expectations of a rewarding ‘warm glow’ after helping another individual (Harbaugh et al., 2007). As previous studies have shown that neural activity associated with reward processing (e.g. NAcc activity) continues to change across adolescence and adulthood (Nelson et al., 2016; Schreuders et al., 2018) it was surprising that there was no evidence in this thesis for reward related processes during prosocial behavior in childhood. It is possible that prosocial behavior might have different underlying motivations in childhood and adulthood, even when expressions of prosocial behavior do not necessarily differ across development (Do, McCormick, & Telzer, 2019). This might be a reflection of the distinct social goals to be achieved in various developmental stages (Nelson et al., 2016). Adults might be more motivated to pursue interpersonal goals (as mirrored in increased activity in NAcc; Mobbs et al. (2009)), whereas children might be more driven to follow social norms (McAuliffe et al., 2017).

Neural signature of social exclusion

The second main finding is that the neural responses to social exclusion in middle childhood are similar to neural responses in older individuals. That is to say, in **chapter 2** and **3** I found that children show increased activity in IFG, sgACC, smPFC and amygdala when they were briefly excluded from a virtual interaction. These patterns of activation are in line with earlier studies that investigated neural reactions to periods of social exclusion in adolescence and adults (Cacioppo et al., 2013; Rotge et al., 2015; Vijayakumar et al., 2017). The commonalities between earlier research and the findings from this thesis are striking given the differences between the set-up of paradigms used to investigate social exclusion. That is to say, in the current set of studies children were only excluded for short intervals (compared to longer periods) and their exclusion coincided with the exclusion from another player (instead of being the only excluded player in the game). The findings of a consistent neural response to short-term social exclusion across two studies therefore touches on the saliency of this negative social event. On a similar note, I found that being included in the virtual game resulted in increased activity in dACC, bilateral insula and striatum, regions that have previously been associated with appraisal and reward processing (Delgado, 2007; Shenhav et al., 2016). Earlier work on neural responses to receiving positive feedback revealed similar activation in dACC and insula (Achterberg et al., 2016; Dalgleish et al., 2017) as well as in striatal regions (Davey et al., 2010), suggesting that both explicit (i.e. positive feedback) and implicit (i.e. inclusion in a game) positive evaluation by peers is processed by regions in the social brain that play a role in affective processing.

Although it should be noted that the attribution of reward processing was derived via reverse inferencing, it is interesting to point out that reward processing in a social situation might play different roles for children and adults. That is to say, being included in the game results in activation of reward processing regions in children (**chapter 3** and **4**), whereas including someone else in the game resulted in reward related activity in adults (**chapter 2**). Speculatively, these findings might reflect a developmental shift in self- and other processing (Crone, 2013; van den Bos, van Dijk, Westenberg, Rombouts, & Crone, 2011): whereas children might experience feelings of reward for themselves but not for others, adults might have increased their socio-cognitive skills to the level of experiencing feelings of reward for vicarious inclusion (Braams et al., 2013).

To better understand how neural responses to social exclusion and inclusion interact with children's social competence, it is necessary to combine a neuroimaging approach with real-life measures of social functioning, such as sociometric measures. For example, even though the (Prosocial) Cyberball Game is a well-validated instrument that has been reliably used to measure the experience of social exclusion (Hartgerink, van Beest, Wicherts, & Williams, 2015; Vijayakumar et al., 2017), it is a reduction of the complexity and intricacies of the actual social environment. Using sociometry to quantify indicators of social competence such as friendship quality (Berndt, 2002) and social acceptance (for review see Gifford-Smith and Brownell (2003)), it would be possible to identify individuals with higher or lower social competence and to find out whether these individual differences are reflected in underlying neural responses to social exclusion.

Heritability of social competence and associated neural processes

Finally, I investigated genetic and environmental influences on social competence and associated neural processes. Behavioral genetics analyses showed no influence of genetics or shared environment on prosocial behavior in a task-situation (**chapter 4)**, whereas parent-reported prosocial behavior was influenced by a combination of genetics and unique environment (**chapter 5)**. These findings are in line with earlier research on the heritability of prosocial behavior (Knafo-Noam et al., 2015; Knafo & Plomin, 2006). Together these findings might illustrate that general trait-like tendencies for prosocial behavior are more strongly driven by genetic factors. This notion fits with the theory that some individuals have a "prosocial personality" that accounts for prosocial behavior in multiple contexts (Eisenberg et al., 2014). As prosocial behavior also has been shown to be heavily influenced by contextual factors (Dunfield & Kuhlmeier, 2013; van Hoorn, van Dijk, Meuwese, Rieffe, & Crone, 2016; van Ijzendoorn, Bakermans-Kranenburg, Pannebakker, & Out, 2010) it is possible that state-like prosocial behavior is more strongly influenced by unique environmental factors.

The division of genetic and environmental influences on state- and trait-like prosocial behavior might be reflected in the neural correlates of prosocial behavior. In **chapter 4**, I showed that neural activity in the precuneus and bilateral insula during acts of prosocial behavior were best explained by unique environmental influences. In contrast, in **chapter 5** I found that surface area of social brain regions (mPFC, pSTS and precuneus) was strongly influenced by genetic factors. Cortical thickness was influenced by both genetic and shared environmental factors. Here brain activity can be seen as a state-like neural correlate, as it is relatively susceptible to temporary influences (e.g. sleep deprivation, see Krause et al. (2017)), whereas brain structure can be seen as a trait-like neural correlate that is stable across contexts and more strongly driven by genetic factors. This distinction is also evident in test-retest reliability research, with lower test-retest reliability estimates for brain function than for brain structure (Heckendorf, Bakermans-Kranenburg, van Ijzendoorn, & Huffmeijer, 2019; Herting, Gautam, Chen, Mezher, & Vetter, 2018; Madan & Kensinger, 2017). It is noteworthy that I also found a negative association between parent-reported empathy and cortical thickness of precuneus, which was in turn influenced by genetic and unique environmental factors. In fact, to a small extent overlapping genetic and environmental factors accounted for variance in empathy and brain structure. Although further research is necessary to confirm this hypothesis, it might be the case that the stable behavioral and neural correlates of prosocial behavior are at least partly driven by similar genetic factors.

With regard to the neural processes underlying social exclusion and inclusion, I found evidence of genetic influence on the processing of social exclusion in **chapter 4**. Specifically, behavioral genetic analyses revealed that activity in smPFC and IFG were partly driven by genetic factors. Activity in other regions associated with social exclusion, as well as in regions associated with social inclusion, showed predominant influences of unique environment. These findings might be interpreted to indicate that even though humans have a fundamental need to belong and to be included in a social context (Baumeister & Leary, 1995), individual reactions to social exclusion are strongly dependent on personal experiences (van Harmelen et al., 2014; van Schie et al., 2017) and therefore influenced by unique environmental factors. As a final note, when discussing outcomes of behavioral genetic analyses we should keep in mind that in the current statistical models unique environment cannot be distinguished from measurement error (Plomin, 2011). Although attempts have been made to disentangle these two factors (see for example Ge et al. (2017)), this should remain a point of focus for future studies.

Future directions

The findings presented in this thesis provide a starting point for future studies to address several exciting questions. In this next section I will first discuss the importance of measuring a broad concept of prosociality, followed by options to investigate specific windows in development. Finally, I will discuss the need for integrating multiple methods.

The concept of prosociality

In this thesis I showed that measuring prosocial behavior with an experimental paradigm or via parent-report resulted in very different estimates of genetic and environmental influences. This highlights the diversity in prosocial behaviors, and the methodological challenges involved in capturing this complex behavior (also see El Mallah (2019) for review). Although some studies have tried to integrate different components of prosocial behavior into a coherent construct (see for example (Knafo-Noam et al., 2015)) we are only beginning to understand how prosocial behavior is presented across contexts and how different motivations might increase prosocial in one situation, but decrease prosocial behavior in another situation. In the current set of studies the relationship between different measures of prosocial behavior was not investigated. Underlying motivations for prosocial behavior were also not taken into account, making it difficult to understand why individuals showed prosocial behavior in different contexts. To correctly assess prosocial behavior in an individual I suggest that multiple assessments (i.e. self-report, other-report, behavioral observation, experimental paradigm) are combined in order to measure prosocial behavior across contexts and recipients. In addition, future research should aim to identify underlying common factors in various prosocial behaviors, with options including empathy (Eisenberg et al., 2006) and self-regulation (Steinbeis & Crone, 2016). Together, these measurements might increase our understanding of the multidimensionality of prosocial behavior.

Developmental windows

The set of studies discussed in this thesis were cross-sectional. Although findings from childhood and adulthood could be compared, the current set of studies did not allow for the exploration of developmental trajectories. For example, it is not possible to draw conclusions about the direction of the brain-behavior relationship (i.e. does neural activity predict prosocial behavior). In order to fully understand the developmental importance of middle childhood it is therefore necessary to investigate the transition from middle childhood into adolescence using longitudinal designs. Adolescence has often been highlighted as a period of tremendous changes, in personal identity as well as in social behavior (Blakemore & Mills, 2014; Crone & Dahl, 2012). Many studies on adolescence now include 9-10 year olds as a younger control group, but pubertal hormonal changes might already have started in that age range (Dorn & Biro, 2011). Instead I propose that children as young as 6-8 years old (i.e. prior to or coinciding with the hormonal preparations for puberty known as adrenarche; Campbell, 2011) should be included in order to fully capture the transition from middle childhood into adolescence on both a behavioral and neural level. Longitudinal designs can also shed a light on the continued importance of achieving social competence in childhood, by investigating how socially competent children become socially competent adolescents, and what external factors might influence individual's trajectories. Information gathered through these studies, aided by longitudinal behavior genetics designs, might tell us which developmental period is the most sensitive for environmental influences, so intervention programs and trainings can be devised accordingly.

Integrating methodologies

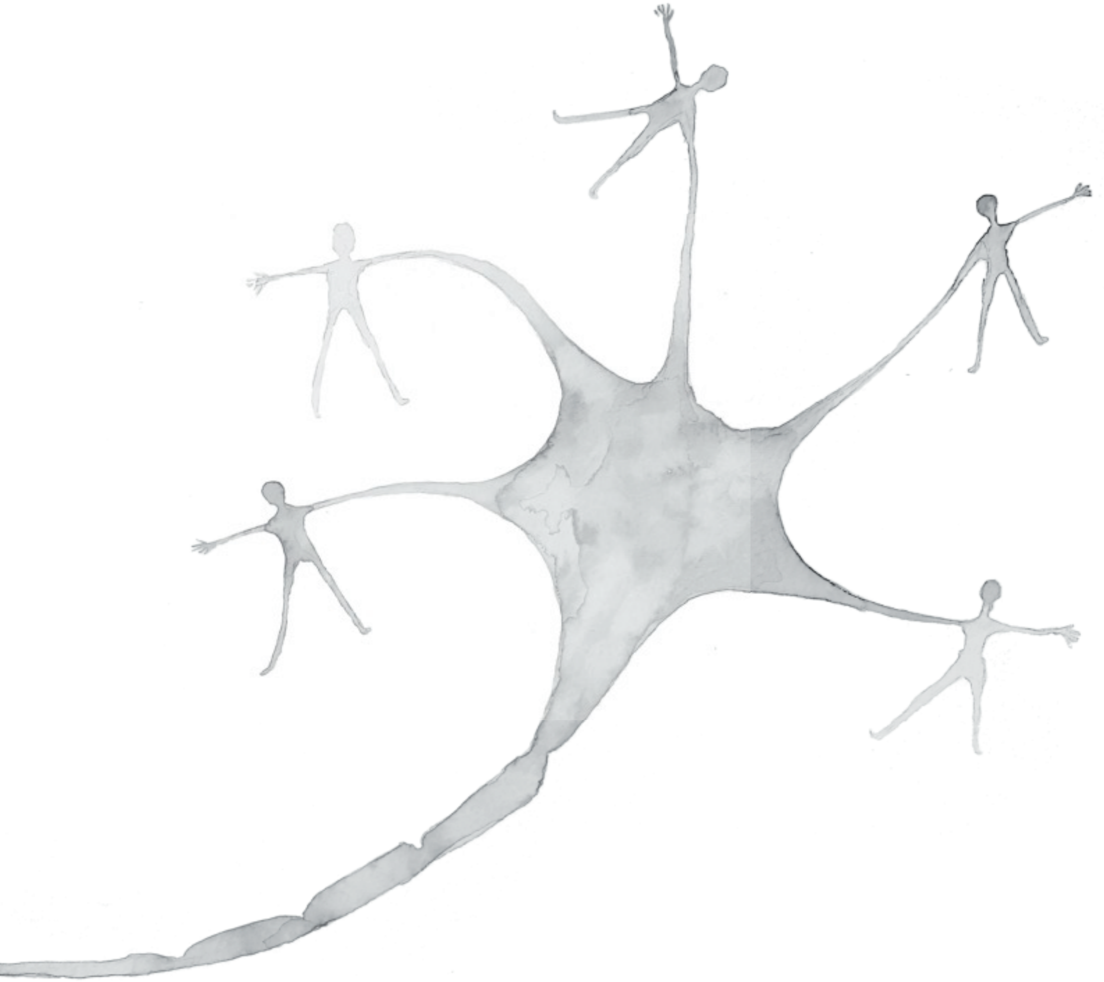
Finally, future research should focus on increasing the integration of various measurements to further our understanding of complex constructs such as social competence. For example, although many studies have investigated the link between brain function and social behavior, the link between brain structure and social competence has not been investigated as extensively. For example, findings from this thesis showed that prosocial behavior results in increased activity of the precuneus, and that higher empathy is associated with lower cortical thickness, but I did not investigate the association between brain functioning and brain structure. Therefore the current set of studies cannot provide an answer to the question whether lower or higher cortical thickness is also associated with different levels of neural activity. If we want to increase our understanding of how the brain-behavior relationship actually works, multi-modal studies combining functional and structural neuroimaging and links to real-life social behavior are necessary (for example see Morishima, Schunk, Bruhin, Ruff, and Fehr (2012); Will, Crone, van Lier, and Güroğlu (2016)). This will also allow us to translate findings from more fundamental research (as described in this thesis) to practical implications for society.

Conclusions

This thesis focused on the heritability of behavioral and neural processes involved in social competence in middle childhood. I investigated how individuals fulfill *others'* and their *own social needs* by studying behavior as well as the structure and functioning of the social brain (Blakemore, 2008). This thesis builds upon the existing literature by demonstrating that children and adults take *others' needs* into account by showing prosocial behavior as a response to observed social exclusion. Additionally, I demonstrated that focusing on *others'* and *own needs* was associated with an integrated set of brain regions involved in socio-cognitive and affective processing. Although extensive neuroimaging research revealed that the structure of the social brain continues to develop from childhood into adulthood (Blakemore, 2008, 2012; Mills et al., 2014) I now show that the functional architecture underlying social competence is already well established in middle childhood. Results on heritability of social competence help us to understand that individual differences in prosocial tendencies and brain structure might be partly influenced by genetic factors, but that neural and behavioral responses in a specific social context are largely shaped by an individual's experiences in the (social) environment. By providing a neurobiological perspective this thesis has highlighted middle childhood as a window of opportunity to increase social competence through training or intervention, and to possibly serve as the starting point for establishing and maintaining of positive social relationships across the lifespan.

APPENDICES

Nederlandse Samenvatting



Introductie

De sociale relatie die we vormen met individuen om ons heen is een fundamenteel onderdeel van de menselijke ontwikkeling. Mensen met een sterk sociaal netwerk leven langer en zijn gelukkiger dan mensen die zich eenzaam voelen (Goswami, 2012; Holt-Lunstad et al., 2010). Om een sociaal netwerk op te kunnen bouwen is het van groot belang dat kinderen al vanaf jonge leeftijd sociale competentie ontwikkelen, oftewel de vaardigheid om zowel eigen als andermans behoeftes in een sociale context te vervullen (Rubin & Rose-Krasnor, 1992). Kinderen kunnen hun eigen en andermans behoeftes vervullen door prosociaal gedrag te laten zien, zoals het helpen van een ander in nood (Eisenberg et al., 2006). Als kinderen echter worden buitengesloten van sociale interactie worden niet alleen hun eigen behoeftes genegeerd, maar lukt het hen wellicht ook niet om de sociale competentie te ontwikkelen die zij later nodig zullen hebben (Rubin et al., 2006).

Het ontwikkelen van sociale competentie wordt onder andere mogelijk gemaakt door de architectuur van het menselijke brein, dat zich heeft aangepast om te voorzien in de behoefte aan sociale interactie (Dunbar & Shultz, 2007). Verschillen in sociale competentie, evenals in de neurale processen die daarmee samenhangen, wordt toegeschreven aan de interactie tussen genen en omgeving (Ebstein et al., 2010). Omdat de basisschoolleeftijd (6-12 jaar) wordt gezien als een cruciale periode in de vorming van sociale competentie is het belangrijk om beter te begrijpen waarom sommige kinderen van deze leeftijd betere sociale vermogens hebben en hoe verschillen tussen kinderen samenhangen met verschillen in onderliggende neurale processen. Het doel van dit proefschrift is dan ook om de processen in sociale competentie gedurende de kindertijd te onderzoeken.

Sociale competentie in de kindertijd

De kindertijd, en specifiek de basisschoolleeftijd (6-12 jaar), is een belangrijke periode in de ontwikkeling van sociale competentie (Del Giudice et al., 2009). Zo ontdekken kinderen in deze ontwikkelingsfase hun persoonlijke interesses en voorkeuren, en worden zij steeds beter in het controleren van hun eigen gedrag en het begrijpen van hun eigen emoties (Del Giudice, 2014; Mah & Ford-Jones, 2012; Steinbeis et al., 2014). Deze persoonlijke ontwikkelingen vallen samen met sociale ontwikkelingen zoals een beter begrip en gebruik van sociale normen, zoals eerlijkheid (Jambon & Smetana, 2014; McAuliffe et al., 2017). Daarnaast ontwikkelen kinderen complexere sociaal-cognitieve en affectieve vaardigheden die nodig zijn om adequaat te kunnen reageren op de behoeftes van een ander. Affectieve vaardigheden omvatten het vermogen om empathisch te zijn en om andermans emoties te begrijpen (Eisenberg et al., 2015; Reschke et al., 2017). Sociaal-cognitieve vaardigheden omvatten het vermogen om het perspectief van een ander in te nemen en het eigen gedrag te kunnen reguleren (Penner & Finkelstein, 1998; Steinbeis, 2018).De combinatie van persoonlijke en sociale ontwikkeling zorgt ervoor dat kinderen sociale competentie kunnen ontwikkelen gedurende deze levensfase, waardoor zij op hun persoonlijke en andermans sociale behoeftes kunnen inspelen.

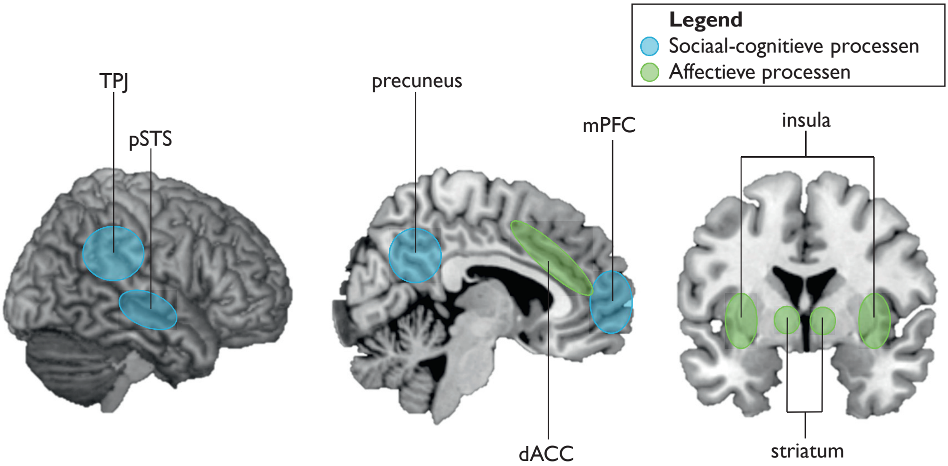
Een mogelijkheid om aan andermans sociale behoeftes te voldoen is door het tonen van *prosociaal gedrag*, wat wordt gezien als een basis voor positieve en wederkerige relaties (Over, 2016). In het kort kan het gedefinieerd worden als vrijwillig gedrag om een ander te helpen (Eisenberg et al., 2006; Padilla-Walker & Carlo, 2014), maar het omvat vele verschillende gedragingen zoals het delen van bezittingen, samenwerken of troosten. Prosociaal gedrag hangt samen met verbeterde academische prestaties en verminderd externaliserend gedrag (Caprara et al., 2000; Padilla-Walker et al., 2015), waardoor de voordelen van prosociaal gedrag ook buiten de sociale context tot uiting komen. Hoewel prosociaal gedrag al op jonge leeftijd getoond kan worden, kunnen uitingen van prosociaal gedrag per context verschillen (Carlo & Randall, 2002; Warneken & Tomasello, 2006). Zo kan iemand geld doneren aan een goed doel, maar meer moeite hebben met het troosten van een onbekende andere. Daarnaast kunnen de motivaties om prosociaal te zijn variëren van altruïstisch (zonder voordeel voor jezelf) tot egoïstisch (omdat je er zelf beter van wordt (Eisenberg & Spinrad, 2014)). Door deze grote diversiteit in gedragingen is er nog veel discussie onder onderzoekers over de beste manier om prosociaal gedrag te meten (zie El Mallah (2019) voor een review). Daarom worden in dit proefschrift twee verschillende meet methodes gehanteerd: prosociaal gedrag wordt gemeten met een experimentele taak en door ouder-rapportage.

Kinderen kunnen hun sociale competentie verder ontwikkelen als zij een positieve band met hun leeftijdsgenoten hebben, maar kinderen die toch al minder sociale competentie hebben lopen het risico buitengesloten te worden en hierdoor niet in hun persoonlijke sociale behoeftes te kunnen voorzien (Rubin et al., 2006). *Sociale buitensluiting* leidt vaak tot internaliserende of externaliserende problemen en gevoelens van eenzaamheid en depressie (Boivin et al., 1995; Ladd, 2006; Ladd & Troop-Gordon, 2003). Kinderen die buitengesloten worden verbergen hun emoties vaak, of ze varen uit tegen de kinderen die hen buitensluiten, waardoor ze ingaan tegen de sociale normen en nog meer reden geven tot buitensluiting. Kinderen die te maken hebben gehad met sociale buitensluiting hebben ook meer kans om later in hun leven sociaal buitengesloten te worden (Hardy et al., 2002). Hierdoor wordt hen mogelijk de kans ontnomen om hun sociale competentie ten volle te ontwikkelen.

Sociale competentie en het brein

Om verschillen in sociale competentie beter te begrijpen zijn in de laatste decennia structurele en functionele *magnetic resonance imaging* (MRI) methodes gebruikt om onderliggende neurale processen in sociale competentie te onderzoeken. Structureel MRI onderzoek heeft aangetoond dat zowel neuronen (grijze stof) als de connecties tussen neuronen (witte stof) in het brein blijven toenemen gedurende de kindertijd. Gedurende de adolescentie (12-25 jaar) worden de belangrijke verbindingen in het brein behouden en verbeterd, terwijl overbodige verbindingen verdwijnen (Wierenga et al., 2014). Dit betekent dat het maximale volume van het brein reeds bereikt wordt in de kindertijd, maar dat de ontwikkeling van het brein nog verder daarna doorloopt.

In functioneel MRI onderzoek onder adolescenten en volwassenen is een netwerk van breingebieden ontdekt dat bekend staat als het sociale brein. Dit netwerk lijkt ontwikkeld te zijn om de complexe functies die nodig zijn voor menselijke interactie te ondersteunen (Blakemore, 2008; Frith & Frith, 2003). Binnen dit sociale brein zijn er gebieden te onderscheiden die een rol spelen in de sociaal-cognitieve en affectieve processen van sociale competentie (zie ook Figuur 1).



Figuur 1. *Visualizatie van het sociale brein. Gebieden gemarkeerd met blauw worden geassocieerd met sociaal-cognitieve processen; temporele-parietale junctie (TPJ), posterieure superieure temporele sulcus (pSTS), precuneus, en mediale prefrontale cortex (mPFC). Gebieden gemarkeerd met groen worden geassocieerd met affectieve processen; dorsale anterieure cingulate cortex (dACC), anterieure insula (AI), en het ventraal striatum (VS). Figuur gebaseerd op Will en Guroglu (2016).*

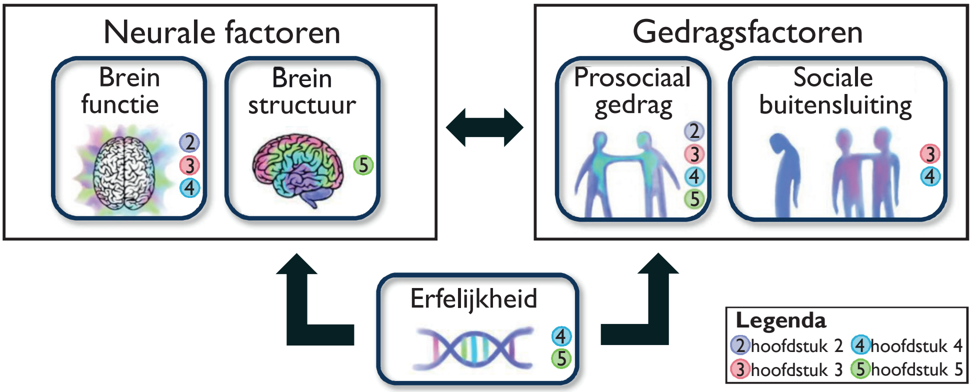
Sociaal-cognitieve processen, zoals het vermogen om het perspectief van een ander aan te nemen, worden geassocieerd met gebieden zoals de mediale prefrontale cortex (mPFC), temporele-parietale junctie (TPJ), posterieure superieure temporele sulcus (pSTS) en precuneus (Blakemore, 2008). Deze gebieden vervullen verschillende functies binnen sociale competentie en in het bijzonder binnen prosociaal gedrag gedrag. Zo is de TPJ belangrijk voor het aannemen van het perspectief van een ander en speelt de precuneus een rol in het ophalen van sociale informatie (Carter & Huettel, 2013; Pfeifer et al., 2007). Affectieve processen, zoals het verwerken van emoties, worden geassocieerd met gebieden zoals de anterieure insula (AI), dorsale anterieure cingulate cortex (dACC) en het ventraal striatum (VS). AI en dACC spelen een belangrijke rol in het opmerken en verwerken van zowel positieve als negatieve sociale gebeurtenissen (Davey et al., 2010; Eisenberger et al., 2003), terwijl het VS vooral geassocieerd wordt met het verwerken van beloningen (Delgado, 2007). Eerder onderzoek heeft aangetoond dat zowel het ervaren als het observeren van sociale buitensluiting geassocieerd is met een toename in neurale activiteit in AI en dACC (Cacioppo et al., 2013), wat aangeeft dat affectieve processen een rol spelen bij zowel persoonlijke als andermans sociale buitensluiting. Gezamenlijk bieden deze eerdere bevindingen een belangrijk uitgangspunt om onderliggende neurale processen voor sociale competentie in de kindertijd te onderzoeken.

Erfelijkheid van sociale competentie

Naast onderzoek naar neurale processen kan sociale competentie ook onderzocht worden door erfelijkheid van dit gedrag in kaart te brengen. Door gebruik te maken van gedragsgenetische analyses kan worden uitgezocht of verschillen in sociale competentie toe te schrijven zijn aan genetische factoren, omgevingsfactoren, of een combinatie van beiden. Kort gezegd omvatten genetische factoren variaties in genen en kunnen omgevingsfactoren opgedeeld worden in gedeelde (b.v. ouderschap of de thuisomgeving) en unieke (d.w.z. specifiek voor een individueel kind) factoren (McLoughlin et al., 2007). Schattingen van erfelijkheid kunnen gemaakt worden in steekproeven met tweelingen, waarin kinderen met 100% gedeelde genen (ééneiige tweelingen) vergeleken kunnen worden met kinderen met 50% gedeelde genen (twee-eiige tweelingen). Als het gedrag van ééneiige tweelingen sterker samenhangt dan het gedag van twee-eiige tweelingen is dit een indicatie van een sterke genetische invloed. Als er een vergelijkbare samenhang is in het gedrag van één- en twee-eiige tweelingen is dit juist een aanwijzing van sterke invloed van de gedeelde omgeving. Als de tweelingen helemaal niet op elkaar lijken in hun gedrag, dan kan de variantie in gedrag waarschijnlijk verklaard worden door unieke omgevingsfactoren of meetfouten (Knafo-Noam et al., 2018). Deze methode heeft eerder uitgewezen dat ongeveer de helft van de verschillen in prosociaal gedrag verklaard kan worden door genetische factoren (Knafo-Noam et al., 2015). Ook verschillen in hersenstructuur bleken grotendeels toe te schrijven aan genetische factoren (Peper et al., 2007), maar vooralsnog is er niet duidelijk in hoeverre neurale activiteit in het brein in een sociale context gedreven wordt door genetische factoren.

Dit proefschrift

Het doel van dit proefschrift was om sociale competentie in de kindertijd in een neurobiologisch perspectief te zetten. Dit proefschrift omvat de resultaten van vier empirische studies, uitgevoerd met een combinatie van gedragsonderzoek, hersenonderzoek en gedrags-genetische analyses, om invloeden van genen en omgeving op sociale competentie en onderliggende neurale processen te onderzoeken. In onderstaand figuur is gevisualiseerd welke methodes in welk hoofdstuk gehanteerd zijn.



Figuur 2. *Visualisatie van de relatie tussen gedrags- neurale en erfelijkheidsfactoren in sociale competentie die in dit proefschrift onderzocht worden.*

De vier empirische hoofdstukken zijn hieronder samengevat. De samenvatting wordt gevolgd door een algemene discussie.

Methode en Resultaten

Om te onderzoeken in welke mate deelnemers prosociaal gedrag vertonen of reageren op sociale buitensluiting, heb ik gebruik gemaakt van een experimentele taak. In de *Prosocial Cyberball Game* (gebaseerd op het werk van Riem, Bakermans-Kranenburg, Huffmeijer, and IJzendoorn (2013)) spelen deelnemers een virtueel balspel met drie geprogrammeerde tegenspelers. De eerste fase van het spel verloopt eerlijk, waarbij de bal naar alle spelers gegooid wordt. In de tweede fase van het spel wordt één van de geprogrammeerde spelers buitengesloten. Hierdoor krijgt de deelnemer de mogelijkheid om *prosociaal gedrag* te vertonen, door relatief vaker naar de buitengesloten speler te gooien en hiermee het buitensluiten te compenseren. Tegelijkertijd kan de deelnemer zich ook zorgen maken over zijn of haar eigen deelname aan het spel, bijvoorbeeld als de deelnemer de bal zelf niet meer krijgt. Door te vergelijken wat er in het brein gebeurd als deelnemers de bal wel of niet krijgen kon ook *sociale buitensluiting* worden onderzocht.

Sociale competentie van volwassenen

In **hoofdstuk 2** heb ik neurale reacties bij het tonen van prosociaal gedrag en bij het observeren van sociale buitensluiting onderzocht. Een groep jongvolwassen vrouwen (N = 23, 18-19 jaar oud) speelde de *Prosocial Cyberball Game* in de MRI scanner. Uit de resultaten bleek dat deelnemers vaker de bal naar de buitengesloten speler gooiden en dus prosociaal gedrag lieten zien. Het gooien naar de buitengesloten speler hing samen met een toename in neurale activiteit in de TPJ, nucleus accumbens (NAcc; onderdeel van het ventraal striatum) en AI. Ook was er een toename in neurale activiteit in de AI terwijl deelnemers sociale buitensluiting observeerden. Deze bevindingen laten zien dat hersengebieden die eerder geassocieerd werden met sociale cognitie en het verwerken van beloningen (respectievelijk de TPJ en NAcc) beiden actief werden tijdens prosociaal gedrag, wat er mogelijk op duidt dat zowel persoonlijke (gevoel van beloning) als ander-gerichte (inleven in een ander) motivaties belangrijk zijn voor prosociaal gedrag. Daarnaast blijkt dat de AI een rol speelt in zowel het tonen van prosociaal gedrag en het observeren van sociale buitensluiting, wat mogelijk erop wijst dat deze gebeurtenissen als betekenisvol werden ervaren. Deze bevindingen vormden een belangrijk startpunt voor mijn onderzoek naar neurale processen in de kindertijd.

Testen en repliceren van bevindingen van sociale competentie in de kindertijd

In **hoofdstuk 3** heb ik sociale competentie in de kindertijd onderzocht. Om ook de repliceerbaarheid van mijn resultaten te controleren heb ik een combinatie van replicatie en meta-analytische methodes toegepast. Drie groepen kinderen (*n* = 18, *n* = 27 en *n* = 26, 7-10 jaar oud) speelden de PCG in de MRI scanner. Gedragsresultaten lieten zien dat kinderen prosociaal gedrag vertoonden na het observeren van sociale buitensluiting van een ander, vergelijkbaar met de bevindingen in **hoofdstuk 2**. Ik vond echter geen significante neurale activiteit in hersengebieden in het sociale brein. Ook uit een meta-analyse over de drie groepen heen bleek geen activiteit in het sociale brein. Bij het onderzoeken van sociale buitensluiting vond ik neurale activiteit in de linker orbitofrontale cortex (OFC) in de drie groepen. Dit gebied werd eerder geassocieerd met het beoordelen van situaties. Als deelnemers de bal ontvingen in het spel (en dus niet werden buitengesloten) vertoonden zij neurale activiteit in de dACC/mPFC, AI en VS, hersengebieden die ook geassocieerd worden met het verwerken van beloningen. Een meta-analyse over de drie groepen heen liet gemeenschappelijke activiteit in de OFC zien gedurende sociale buitensluiting, en gemeenschappelijke activiteit in dACC en VS gedurende mee mogen doen in het spel. Samen suggereren deze resultaten dat sociale buitensluiting in de kindertijd als negatief wordt ervaren, terwijl mee mogen doen in een spel als positief wordt ervaren. Ook laten deze resultaten zien dat de bevindingen in verschillende groepen gerepliceerd konden worden, wat de betrouwbaarheid van deze bevindingen vergroot. De onderliggende neurale mechanismen voor prosociaal gedrag zijn echter niet duidelijk geworden in deze studie, mogelijk door de grote variatie in prosociaal gedrag.

Erfelijkheid van functie van het brein voor sociale competentie

Grote variaties in gedrag kunnen beter onderzocht worden in grotere steekproeven. In **hoofdstuk 4** heb ik daarom de associatie tussen prosociaal gedrag en functioneren van het brein in de kindertijd verder onderzocht in een grote groep tweelingen. Hierdoor stelde deze steekproef mij ook in staat om te onderzoeken in welke mate genen en omgeving invloed hebben op sociale competentie. In dit hoofdstuk onderzocht ik zowel prosociaal gedrag als sociale buitensluiting in de kindertijd (*N* = 512, 7-9 jaar oud). Evenals in **hoofdstuk 3** vertoonden kinderen prosociaal gedrag tijdens het spelen van de PCG. Het tonen van prosociaal gedrag hing samen met een toename in neurale activiteit in de precuneus, een hersengebied wat ook onderdeel uitmaakt van het sociale brein. Daarnaast liet een aanvullende analyse zien dat deelnemers die meer prosociaal gedrag vertoonden in het spel, minder neurale activiteit in de AI lieten zien als zij de bal gooiden naar de buitengesloten speler. Ik heb deze bevinding geïnterpreteerd in het licht van onderliggende processen voor prosociaal gedrag: mogelijk zijn zowel het perspectief van de ander nemen (wat samenhangt met activiteit in de precuneus) als het bewust zijn van sociale normen (wat samenhangt met activiteit in de AI) van belang bij het vertonen van prosociaal gedrag. Gedrags-genetische analyses toonden aan dat de bevindingen voor prosociaal gedrag evenals onderliggende neurale processen beïnvloed werden door unieke omgevingsfactoren en/of meetfouten, wat duidt op de sterke invloed van de specifieke context op het tonen van prosociaal gedrag.

Mijn tweede set resultaten liet zien dat het ervaren van sociale buitensluiting samenhing met een toename in neurale activiteit in de superieure mediale prefrontale cortex (smPFC), inferieure frontale gyrus (IFG) en subgenuale ACC (sgACC). Mee mogen doen in het spel hing samen met een toename in neurale activiteit in de AI, bilateral putamen en dACC. Neurale activiteit in de smPFC en IFG bleek beïnvloed te worden door een combinatie van genetica en unieke omgevingsfactoren. Activiteit in de andere gebieden werd beïnvloed door unieke omgevingsfactoren en/of meetfouten. Samengevat laten de resultaten uit dit hoofdstuk zien dat de neurale processen die samenhangen met prosociaal gedrag en sociale buitensluiting voornamelijk beïnvloed worden door individuele ervaringen, zoals te zien in de sterke invloed van unieke omgevingsfactoren. Genetische factoren spelen slechts een kleine rol in de neurale processen omtrent sociale buitensluiting.

Erfelijkheid van structuur van het brein voor sociale competentie

Als toevoeging op mijn onderzoek naar erfelijkheid van de functie van het brein heb ik onderzocht in hoeverre de structuur van het sociale brein beïnvloedt wordt door genetische en omgevingsfactoren. Hiervoor heb ik in **hoofdstuk 5** onderzocht hoe de oppervlakte en dikte van de hersenschors van vier vooraf geselecteerde gebieden (mPFC, TPJ, pSTS en precuneus) van 7-9 jarige kinderen (*n* = 486) werd beïnvloed door genetische en omgevingsinvloeden. Ook heb ik onderzocht in hoeverre prosociaal en empathisch gedrag van de kinderen in verschillende contexten (gemeten via ouder-rapportage) werden gedreven door genetische en omgevingsfactoren. De gedrags-genetische analyses wijzen uit dat genetische factoren een belangrijke invloed zijn op oppervlakte van de mPFC, pSTS en precuneus. Oppervlakte van de TPJ leek echter door zowel genetische als gedeelde omgevingsfactoren beïnvloed. Ook voor dikte van de hersenschors van de precuneus bleken genetische factoren een belangrijke rol te spelen. Voor de anderen gebieden leken zowel genetische als gedeelde omgevingsfactoren invloed te hebben op de dikte van de hersenschors. Deze bevindingen laten zien dat de hersengebieden in het sociale brein niet alleen sterk beïnvloed worden door genetische factoren, maar ook relatief meer beïnvloed worden door gedeelde omgevingsfactoren, in vergelijking met andere hersengebieden. Deze bevinding suggereert dat de (sociale) omgeving een sterkere invloed heeft op hersengebieden in het sociale brein dan op hersengebieden buiten het sociale brein. Zowel prosociaal gedrag en empathie van de kinderen bleken sterk beïnvloed door genetische factoren.

Een tweede onderzoeksvraag binnen dit hoofdstuk was in welke mate genetische en omgevingsfactoren zouden overlappen in hun invloed op zowel structuur van het brein als op prosociaal gedrag. De resultaten lieten zien dat er een kleine overlap was in genetische en unieke omgevingsfactoren voor de dikte van de hersenschors van de precuneus en empathisch gedrag, wat betekent dat bepaalde genetische en unieke omgevingsfactoren ervoor zorgen dat sommige kinderen zowel een dunnere hersenschors hebben als een hogere mate van empathie. Ook lieten de resultaten zien dat een aantal genetische en unieke omgevingsfactoren invloed hebben op zowel prosociaal gedrag als empathie, wat betekent dat deze twee gedragingen deels door dezelfde factoren beïnvloed worden maar dat er ook unieke factoren een rol spelen. Samengevat laten deze bevindingen zien dat zowel de structuur van het sociale brein als van prosociaal en empathisch gedrag sterk wordt beïnvloed door genetische invloeden, maar dat slechts een klein deel van deze invloeden overlapt voor brein en gedrag.

Algemene Discussie

Gezamenlijk kunnen de studies beschreven in dit proefschrift worden samengevat in een aantal belangrijke uitkomsten. Ik bespreek hier eerst de resultaten met betrekking tot functie en structuur van het brein en benoem daarna de belangrijkste resultaten met betrekking tot erfelijkheid. Vervolgens noem ik een aantal aanbevelingen voor vervolgonderzoek.

De rol van het brein in sociale competentie

Allereerst toonde ik aan dat het observeren van sociale buitensluiting *prosociaal gedrag* uitlokt in zowel volwassenen (**hoofdstuk 2**) als kinderen (**hoofdstuk 3** en **4**). Dit prosociale gedrag hing samen met een toename in neurale activiteit in de TPJ (in volwassenen) en de precuneus (in kinderen), hersengebieden die eerder geassocieerd werden met sociaal-cognitieve processen (Blakemore, 2008). Dit suggereert dat proberen voor te stellen hoe een ander zich voelt belangrijk is voor het vertonen van prosociaal gedrag. Bijzonder interessant is de bevinding dat de precuneus zowel op functionele (**hoofdstuk 4**) als op structurele (**hoofdstuk 5**) maten van het brein samenhangt met prosociaal gedrag in de kindertijd: dit toont aan dat de precuneus een rol speelt in prosociaal gedrag in een specifieke context zoals bij het observeren van sociale buitensluiting, maar ook in verschillende contexten. Naast sociaal-cognitieve processen bleken ook sociaal-affectieve processen een belangrijke rol te spelen in het vertonen van prosociaal gedrag. Ik toonde aan dat kinderen die minder prosociaal gedrag vertoonden een verhoogde neurale activiteit in de AI lieten zien bij het gooien van de bal naar de buitengesloten speler. Aangezien de AI een belangrijke rol speelt in het overschrijden van sociale normen (Güroğlu et al., 2011) geeft deze neurale activiteit mogelijk aan dat kinderen zich bewust zijn van de sociale norm om een ander in nood te helpen. Bij het gooien van de bal naar de buitengesloten speler vertoonden volwassen juist verhoogde activiteit in de NAcc. Deze bevinding past bij de hypothese dat prosociaal gedrag een warm gevoel oplevert (Harbaugh et al., 2007). Gezien de verschillende neurale bevindingen bij volwassenen en kinderen is het mogelijk dat motivaties voor prosociaal gedrag veranderen gedurende de ontwikkeling, zelfs als het prosociale gedrag niet verschillend is voor kinderen en volwassenen (zie ook Do et al. (2019)). Mogelijk is voor kinderen het volgen van sociale normen heel belangrijk (McAuliffe et al., 2017), maar worden volwassenen ook intrinsiek gemotiveerd om iets aardigs te doen voor een ander (Mobbs et al., 2009).

Ten tweede toonde ik aan dat kinderen verhoogde neurale activiteit in een netwerk van IFG, sgACC, smPFC en amygdala lieten zien bij *sociale buitensluiting*. Opvallend is dat neurale reacties op korte periodes van buitensluiting in de kindertijd (zoals in dit proefschrift) hetzelfde zijn als neurale reacties op lange periodes van buitensluiting, zoals eerder onderzocht in adolescenten en volwassenen (Cacioppo et al., 2013; Rotge et al., 2015; Vijayakumar et al., 2017). Hieruit blijkt dat zowel korte als lange periodes van sociale buitensluiting worden ervaren als een saillante gebeurtenis vanaf de kindertijd tot in de volwassenheid. Aanvullend vond ik dat *mee mogen spelen in het balspel* samenhing met verhoogde neurale activiteit in dACC, bilaterale anterieure insula en het striatum. Eerder onderzoek naar neurale reacties op positieve feedback liet ook verhoogde neurale activiteit in deze gebieden zien (Achterberg 2016; Dalgleish 2017; Davey 2010). Gezamenlijk suggereren deze resultaten dat neurale gebieden die betrokken zijn bij sociaal-affectieve processen reageren op zowel expliciete (b.v. positieve feedback) als impliciete (b.v. mee mogen doen in een spel) sociale evaluaties van leeftijdsgenoten. Het is interessant om op te merken dat verschillende sociale processen mogelijke gevoelens van beloning oproepen bij kinderen en volwassenen. Zo lieten kinderen verhoogde activiteit in het striatum zien bij het ervaren van sociale inclusie, terwijl volwassenen verhoogde activiteit in het striatum lieten zien bij het vertonen van prosociaal gedrag. Wellicht laten deze bevindingen een ontwikkeling zien in het nadenken over zelf en anderen (Crone, 2013; van den Bos et al., 2011): kinderen krijgen een gevoel van beloning als het een positieve situatie voor henzelf betreft, terwijl volwassenen ook een gevoel van beloning ervaren als een ander zich in een positieve situatie bevindt (Braams et al., 2013).

Erfelijkheid van sociale competentie

Als laatste vond ik verschillende invloeden van genetica en omgeving op prosociaal gedrag: prosociaal compensatie gedrag in de PCG werd beïnvloed door unieke omgevingsfactoren, maar prosociaal gedrag dat door de ouders van de kinderen werd gerapporteerd bleek beïnvloed door een combinatie van genetische en unieke omgevingsfactoren. Deze bevindingen benadrukken de complexiteit en multidimensionaliteit van prosociaal gedrag, en laten zien dat verschillende maten van prosociaal gedrag tot verschillende conclusies kunnen leiden. Aan de ene kant zijn sommige mensen over het algemeen prosocialer dan anderen: dit zijn mensen die in alle situaties een prosociale reactie zullen vertonen (Eisenberg et al., 2014). Dit stabiele prosociale gedrag kan beter worden gemeten door vragenlijsten in te laten vullen en wordt mogelijk sterker beïnvloed door genetica dan door omgevingsfactoren. Aan de andere kant laten sommige mensen hun gedrag sterk afhangen van de situatie waarin zij zich bevinden (b.v. of er anderen aanwezig zijn, hoe zij zich voelen op dat moment; (Dunfield & Kuhlmeier, 2013; Van Hoorn et al., 2016; van Ijzendoorn et al., 2010), waardoor dit type prosociaal gedrag mogelijk sterker beïnvloed wordt door unieke omgevingsfactoren.

Dit onderscheid tussen temporele en stabiele factoren kwam ook naar voren uit mijn resultaten met betrekking tot het brein. Zo vond ik dat neurale activiteit tijdens prosociaal gedrag werd gedreven door unieke omgevingsinvloeden. Aanvullend vond ik dat maten van brein structuur (d.w.z. oppervlakte en dikte van de hersenschors) naast gedeelde en unieke omgevingsinvloeden ook invloeden van genetica lieten zien. Hersenactiviteit kan hier wellicht gezien worden als een temporele staat, die eenvoudig beïnvloed kan worden door tijdelijke veranderingen zoals slapeloosheid (Krause et al., 2017), terwijl hersenstructuur stabiel is in verschillende contexten en daardoor vooral door genetische factoren beïnvloed wordt.

Wat betreft sociale buitensluiting lieten de gedragsgenetische analyses in **hoofdstuk 4** een iets ander plaatje zien: hoewel hersenactiviteit tijdens sociale buitensluiting voor het grootste deel beïnvloed werd door unieke omgevingsfactoren (vergelijkbaar met neurale activiteit tijdens prosociaal gedrag) was er ook een kleine invloed van genetische factoren. Deze bevindingen geven mogelijk aan dat de reactie op sociale buitensluiting deels een fundamentele, genetische basis heeft, omdat het behoren tot een groep voor ieder individu belangrijk is (Baumeister & Leary, 1995). Tegelijk worden reacties op sociale buitensluiting ook deels bepaald door persoonlijke ervaringen (van Harmelen et al., 2014; van Schie et al., 2017) en andere unieke omgevingsinvloeden. In dit proefschrift kon er geen onderscheid gemaakt worden tussen unieke omgevingsinvloeden en meetfout, dus dit moet verder onderzocht worden in vervolgonderzoek om scherpere conclusies te kunnen trekken op het gebied van invloed van unieke omgevingsfactoren.

Aanbevelingen voor vervolgonderzoek

De onderzoeken die beschreven worden in dit proefschrift bieden een basis voor vervolgonderzoek om een aantal interessante vragen uit te werken. Hieronder doe ik een aantal aanbevelingen die naar mijn mening relevant kunnen zijn voor vervolgonderzoek.

Ten eerste toonde ik in dit proefschrift aan dat verschillende methodes om prosociaal gedrag te meten ook verschillende inschattingen van genetische en omgevingsinvloeden opleveren. Om prosociaal gedrag van een individu goed te kunnen meten zouden daarom verschillende metingen (b.v. zelf-rapportage, rapportage van anderen, gedragsobservatie, experimentele paradigma's) gecombineerd moeten worden. Daarnaast zou vervolgonderzoek onderliggende factoren in verschillende prosociale gedragingen in kaart kunnen brengen, zoals empathie (Eisenberg et al., 2006) en zelf-regulatie (Steinbeis & Crone, 2016). Gezamenlijk kunnen deze metingen mogelijk ons begrip van de multidimensionaliteit van prosociaal gedrag vergroten.

Ten tweede moet opgemerkt worden dat de onderzoeken in dit proefschrift cross-sectioneel waren. Hoewel ik wel bevindingen uit de kindertijd en de volwassenheid kon vergelijken, konden ontwikkelingstrajecten niet verder in kaart worden gebracht. Om ten volle te begrijpen welke rol de kindertijd speelt in de sociale ontwikkeling van de mens is het daarom cruciaal om longitudinale studies uit te voeren die bijvoorbeeld de overgang van kindertijd naar adolescentie verduidelijken. Longitudinaal onderzoek kan daarnaast ook inzicht geven in het belang van sociale competentie in de kindertijd, door duidelijk te maken hoe sociaal competente kinderen uitgroeien tot sociaal competente volwassenen, en welke externe factoren deze individuele trajecten kunnen beïnvloeden. Informatie verkregen uit dit type onderzoek, gecombineerd met longitudinale gedrags-genetische onderzoeken, kan ons meer vertellen over welke periode in de ontwikkeling het meest gevoelig is voor omgevingsinvloeden, zodat interventie programma's en trainingen effectief toegepast kunnen worden.

Als laatste stel ik voor dat toekomstig onderzoek de focus zou moeten leggen op het integreren van verschillende meet methodes om ons begrip van complexe constructen zoals sociale competentie te bevorderen. Als we beter willen begrijpen hoe de relatie tussen ons gedrag en ons brein er daadwerkelijk uitzien is het noodzakelijk om multi-modale onderzoeken op te zetten die functioneel en structureel hersenonderzoek combineren met maten van sociaal gedrag in het dagelijks leven (zie bijvoorbeeld Morishima et al. (2012); Will, Crone, van Lier, and Güroğlu (2016)). Dit zal ons ook in staat stellen om de vertaalslag te maken van meer fundamenteel onderzoek (zoals beschreven in dit proefschrift) naar praktische implicaties voor de maatschappij.

Conclusie

In dit proefschrift heb ik onderzocht in hoeverre sociale competentie in de kindertijd beïnvloed worden door genetische en omgevingsfactoren. Ik heb beschreven hoe individuen *andermans* en *hun eigen* sociale behoeftes vervullen door zowel sociaal gedrag als de structuur en de functie van het sociale brein (Blakemore, 2008) te bestuderen. Met dit proefschrift bouw ik voort op bestaande literatuur door aan te tonen dat kinderen en volwassenen *andermans behoeftes* vervullen door prosociaal gedrag te vertonen na het observeren van sociale buitensluiting. Daarnaast liet ik zien dat het vervullen van *andermans* en *eigen behoeftes* samenhangt met neurale activiteit in een set brein gebieden die belangrijk zijn voor sociaal-cognitieve en affectieve processen. Hoewel eerder neuroimaging onderzoek liet zien dat de structuur van het sociale brein blijft ontwikkelen van de kindertijd tot in de volwassenheid (Blakemore, 2008, 2012; Mills et al., 2014) laat ik nu zien dat de functionele architectuur van het brein die belangrijk is voor sociale competentie ook al aanwezig is in de kindertijd. De resultaten met betrekking tot de erfelijkheid van sociale competentie helpen ons begrijpen dat individuele verschillen in prosociale gedragingen en brein structuur deels beïnvloed worden door genetische factoren, maar dat neurale en gedragsmatige reacties in een specifieke sociale context grotendeels gedreven worden door persoonlijke ervaringen van een individu in de (sociale) omgeving. Vanuit een neurobiologisch perspectief laat dit proefschrift zien dat de kindertijd een periode van mogelijkheden voor het ontwikkelen van sociale competentie is, die mogelijk kan dienen als het startpunt voor het tot stand brengen en behouden van positieve sociale relaties gedurende het menselijk leven.

APPENDICES

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APPENDICES

List of Publications

Crone, E.A., Achterberg, A., Dobbelaar, S., Euser, S., van den Bulk, B.G., **van der Meulen, M.**, van Drunen, L., Wierenga, L.M., Bakermans-Kranenburg, M.J., & van IJzendoorn, M.H. Neural and behavioral signatures of social evaluation development in childhood and adolescence: The Leiden Consortium on Individual Development (L-CID). (*Manuscript in preparation, 2019*).

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Peters, S., **van der Meulen, M.**, Zanolie, K., & Crone, E. A. (2017). Predicting reading and mathematics from neural activity for feedback learning. *Developmental Psychology, 53*(1), 149-159.

van der Meulen, M., van IJzendoorn, M. H., & Crone, E. A. (2016). Neural correlates of prosocial behavior: compensating social exclusion in a four-player cyberball game. *PLOS ONE, 11*(7), e0159045.

APPENDICES

Curriculum Vitae

Mara van der Meulen was born on March 22nd in Zoetermeer, the Netherlands. After her graduation from high school (Erasmus College, Zoetermeer) in 2009, Mara obtained her Bachelor's degree in Psychology in 2012 and her Research Master's degree in Developmental Psychology in 2014 from Leiden University. After completing her studies, Mara worked as a research assistant in the Brain and Development Research Center to examine the effect of media-by-peer interactions on body image in young women. In January 2015 Mara started her PhD project in the Brain and Development Research Center at Leiden University, as a part of the Leiden Consortium on Individual Development. Under supervision of Prof. Dr. Eveline Crone she investigated neural processes associated with prosocial behavior and social exclusion in middle childhood. In January 2020 Mara will start working as a post-doctoral researcher at the Leiden Consortium on Individual Development, to investigate longitudinal trajectories of prosocial behavior from childhood into adolescence.