Social behavior in young twins : are fearfulness, prosocial and aggressive behavior related to frontal asymmetry?

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Contents

CHAPTER 1

General introduction

General introduction

Children react to and interact with the world in their own unique way: some children approach new situations with joy whereas others are careful and tend to withdraw. Whether children show adequate approach or withdrawal reactions depends on the situation but also on the temperament and social competences of the child. For example, when someone needs help, it is desirable to show approach behavior, however, some children might be too shy or fearful to help. On the other hand, when someone gives a negative judgment it is often more appropriate to let it go and withdraw but some children react aggressively. Previous studies have shown that approach and withdrawal tendencies can be examined using a specific measure of frontal brain activity, namely frontal asymmetry (FA, Harmon-Jones, Gable, & Peterson, 2010). In this thesis we were specifically interested in FA as a possible neural correlate in relation to fearfulness, prosocial behavior and aggressive behavior. Because we investigated this in a sample of young twins we could also examine heritability of the traits.

It is important to investigate temperamental factors like fearfulness and social behavior like prosociality and aggression in early childhood because these variables have a great impact on social competences later in life (Crick, 1996; Zentner & Shiner, 2015; Buss & Plomin, 2014; Dodge et al., 2003; Buckley, Winkel, & Leary, 2004). In our study, we obtained FA data from 4-6 year old children, an age range that is not often examined in FA research. Most studies include adults or children aged 0-3 years or 6-18 years old (see Coan & Allen, 2004; Reznik & Allen, 2018 for reviews). Especially in recent studies there seems to be a gap of FA research around the age of 4-6 years old, although social behavior plays an important role around the age of four as most children attend school at this age and experience social acceptance and rejection from classmates. Therefore we developed tasks for 4-6 year old children to measure prosocial behavior in reaction to social exclusion of others and to measure aggressive behavior in reaction to social judgments. We hypothesized that relatively greater left frontal brain activity (left FA), related to approach behavior, would be associated to prosocial and aggressive behavior. On the other hand, we hypothesized that relatively greater right frontal brain activity (right FA), related to withdrawal behavior, would be associated to fearfulness.

Furthermore, we hypothesized that an overlap in genetic and/or environmental influences would underlie possible associations between FA and fearfulness. In the following sections we will elaborate on measuring FA and the associations between FA and fearfulness, prosocial behavior and aggressive behavior.

Frontal asymmetry

Research shows that motivational tendencies are related to asymmetric frontal brain activity: relatively greater left frontal brain activity reflects a tendency toward approach behavior and relatively greater right frontal brain activity reflects a tendency toward withdrawal behavior (Davidson, Ekman, Saron, Senulis, & Friesen, 1990; Harmon-Jones et al., 2010; Harmon-Jones & Gable, 2018). This difference in frontal brain activity between the right and left frontal cortex, referred to as Frontal Asymmetry (FA), can be measured with electroencephalography (EEG). EEG is a method to record electrical activity of the brain by placing electrodes along the scalp (see Figure 1A). When the brain signal is amplified, the voltage fluctuations of the electrical currents in the brain cells can be seen as oscillations at various frequencies (faster and slower waves in the ongoing EEG). The magnitude or prominence of activity at a particular frequency is referred to as power and is commonly expressed in either squared microvolts or decibels. The frequency of an oscillation is different under specific situations, for instance when someone is concentrated the frequency of the oscillations is higher than in rest. To compute FA we use the alpha frequency range because higher power in the EEG alpha waves reflects deactivation (Cook, O'Hara, Uijtdehaage, Mandelkern, & Leuchter, 1998; Laufs et al., 2003). Alpha waves are thus oscillations that arise during rest and occur in the frequency range of 8 – 12 Hz in adults. In children the brain in still developing and in 4-6 year old children a frequency range of 6 – 10 Hz resembles the adult frequency range of alpha waves (Marshall, Bar-Heim, & Fox, 2002). As described above, higher alpha power reflects deactivation. With regard to computing FA this means that higher alpha power over the left frontal cortex (compared to the right frontal cortex) reflects deactivation over the left frontal cortex and thus greater activity of the right frontal areas (compared to the left frontal cortex). We will refer to this as right FA. Conversely, higher alpha power over the right than the left frontal cortex

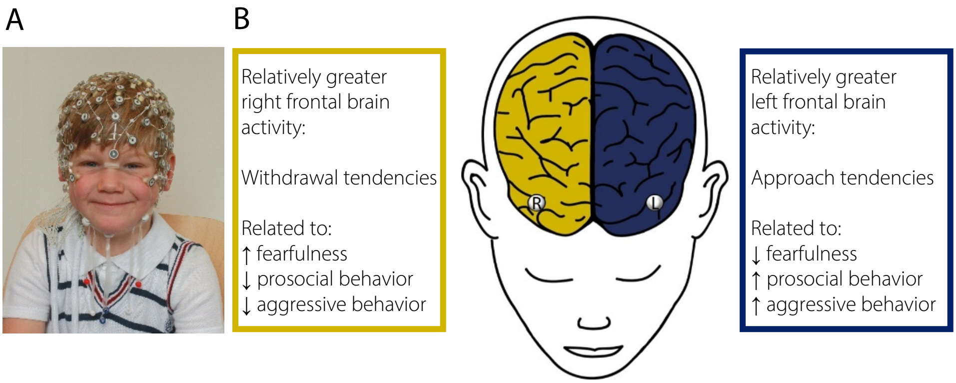

Figure 1. EEG and frontal asymmetry. A) Child wearing an EEG net. B) Visual overview of our hypotheses: the associations between FA and fearfulness, prosocial behavior and aggressive behavior are depicted with arrows. The right site of the brain is presented in yellow and the left site of the brain is presented in blue. FA was measured by using two electrodes which are depicted at the frontal left (L) and right (R) site.

reflects relatively greater activity of the left frontal cortex, which we will refer to as left FA. According to the motivational direction model (Davidson et al., 1990; Harmon-Jones et al., 2010; Harmon-Jones & Gable, 2018) approach behavior is related to left FA and withdrawal behavior is related to right FA. In this thesis we focus on three different characteristics that are related with either approach or withdrawal behavior: fearfulness, prosocial behavior and aggressive behavior, see Figure 1B. FA can be measured both as a state and as a trait. In this thesis we take both approaches into account, for more information see chapter 2 and 3 for state-related FA and chapter 4 for trait-related FA.

Fearfulness

Fear indicates how nervous or worried someone is in relation to anticipated pain, distress or threatening situations. Most individuals tend to avoid frightening situations or stimuli, but some children are more fearful in general than others. This is part of their temperament and known as fearfulness. Because fearfulness usually results in withdrawal behavior we hypothesize that it is related to right FA according to the motivational direction model. Indeed, research has shown that relatively greater right frontal brain activity during rest is related to fearfulness in adults (Mathersul, Williams, Hopkinson, & Kemp, 2008) and children (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Schmidt, 2008). However, developmental samples show inconsistent results for the relation between fear and/or fearfulness and FA (Diaz & Bell, 2012; Howarth, Fettig, Curby, & Bell, 2016; LoBue, Coan, Thrasher, & DeLoache, 2011). A possible explanation for these inconsistent results could be that effortful control, another temperamental factor, is involved in the relation between fearfulness and right FA (see Neal & Gable, 2017). For instance, children who are fearful but can control their fear because of their effortful control may show relatively greater right frontal brain activity but score lower on the fearfulness factor. In Chapter 2 of this thesis we will examine the relation between fearfulness and FA more in depth and investigate whether the two traits show an overlap in their genetic and/or environmental influences. In addition we will take effortful control into account by computing partial correlations between fearfulness and FA with effortful control as covariate. Next to the relation between withdrawal behavior and right FA we are also interested in the relation between approach behavior and left FA, in particular prosocial behavior.

Prosocial behavior

In general, prosocial behavior can be defined as "any action that serves to benefit another person" (Schroeder & Graziano, 2015). In light of the motivational direction model we expect that prosocial behavior is related to approach tendencies because prosocial actions like helping, sharing, comforting or including others often start with approaching the other person. Research indeed showed that prosocial behavior like comforting is related to left FA in infants (Paulus, Kühn-Popp, Licata, Sodian, & Meinhardt, 2013) and prosocial behavior like donating to a charity is related to left FA in adults (Huffmeijer, Alink, Tops, Bakermans-Kranenburg, & Van IJzendoorn, 2012). Prosocial behavior can be measured in various ways, for instance using parental report, which indicates the prosocial behavior of the child in a more general, trait-related way, or with observations and behavioral tasks that are more focused on one specific form of prosocial behavior like helping, sharing or comforting (Paulus, 2018). However, research has shown that these different measures are not strongly correlated with each other, suggesting that prosociality is a multidimensional construct (Paulus, 2018; Padilla-Walker & Carlo, 2015) and that prosocial performance depends on situational factors like probing or modelling (Van IJzendoorn, Bakermans-Kranenburg, Pannebakker, & Out, 2010; Wildeboer et al., 2017). In this thesis we are specifically interested in prosocial behavior in response to perceived social exclusion and the association with left FA. Previous studies in adults, adolescents and older children have shown that most individuals compensate for social exclusion by including the excluded player (Riem, Bakermans-Kranenburg, Huffmeijer, & Van IJzendoorn, 2013; Vrijhof et al., 2016; Van der Meulen, Van IJzendoorn, & Crone, 2016; Van der Meulen et al., 2017). In Chapter 3 we present an adjusted version of this prosocial task for 4-6 year old children: the Prosocial Owl Games (POG). In the POG, two cartoon owls exclude a third owl, and the child can compensate for this exclusion by giving the excluded owl the next turn. We hypothesized that prosocial behavior as measured with the POG is associated with left FA. Next to prosocial behavior we were also interested in the association between left FA and aggressive behavior in response to social judgments in early childhood.

Aggressive behavior

According to the social belongingness hypothesis (Baumeister & Leary, 1995) social acceptance is important for individuals. Being rejected or receiving negative social judgments during childhood may results in mental health problems and higher stress levels later in life (Lereya, Copeland, Costello, & Wolke, 2015; Newman, Holden, & Delville, 2010). Moreover, negative social judgments are associated with anger and sadness that in turn can lead to aggressive behavior (Dodge et al., 2003; Buckley et al., 2004). Previous studies have shown that both adults and 7-10 year old children react more aggressively after a negative social judgment compared to a positive social judgment (Achterberg, van Duijvenvoorde, Bakermans-Kranenburg, & Crone, 2016; Achterberg et al., 2017). In these studies aggressive behavior was measured by blasting noises to the judging peers. After receiving a social judgment, the participant could press a button; the longer the participant pressed the button, the louder the noise was to the judging peer. In Chapter 4 we present an adjusted version of this social judgment task to measure aggressive behavior in early childhood. In the Social

Network Aggression Task for Early Childhood (SNAT-EC), children receive positive, negative and neutral social judgments about their chosen cuddly animal by same-aged unfamiliar peers. Aggressive behavior was operationalized as the duration of a button press with which children could destroy balloons of the judging peer, thus reducing the number of remaining balloons for that peer. In addition, we examined the role of FA in aggressive behavior in 4-6 year old children. Research showed that in adults anger and aggression are related to left FA (see Harmon-Jones et al., 2010 for a review) and more specifically, anger provoked by receiving insults from peers results in left FA (Harmon-Jones & Sigelman, 2001). Therefore we hypothesized that FA might operate as a mediator: negative social judgments result in relatively greater left frontal brain activity and in turn, left FA induces more aggressive behavior as measured with our newly developed task in early childhood.

Setting and design

The studies of this thesis were embedded in a larger, longitudinal twin study: the Leiden Consortium on Individual Development (L-CID). The goal of L-CID is to examine the efficacy of an intervention (the Video-feedback Intervention to promote Positive Parenting and Sensitive Discipline, VIPP-SD, Juffer, Bakermans-Kranenburg, & Van IJzendoorn, 2008) on parenting quality and children's social competences and behavioral control (see for the study protocol Euser et al., 2016). At this moment L-CID consists of two cohorts each including about 250 families, an early childhood cohort with 3-4 year old twins and a middle childhood cohort with 7-8 year old twins at the start of the study. By using two cohorts in an experimental cohort-sequential design with twin families we are able to combine a randomized control trial with an accelerated longitudinal study. The two cohort have overlapping measurements, the children of one cohort will be 7-9 years old during the last two measurements and the children of the other cohort will be 7-9 years old during the first two measurements. This way we obtain data from twin families with twins in the age range of 3-14 years. Families with same-sex monozygotic or dizygotic twins were recruited in the western region of the Netherlands. Each family was invited to take part in the study for six years with yearly visits, either at home or in the laboratory at the university of Leiden. The first two visits were used as baseline assessments and took part before the VIPP-SD intervention. The last four visits serve as post-test assessments. For the current thesis we used data from a pilot study and from the second visit of the early childhood cohort, at this point in time the children were around the age of 4-6 years old. The visits took place at the laboratory of the Leiden University.

Behavioral genetics

Because we included same-sex mono- and dizygotic twins we could examine genetic and environmental influences on the traits, that is, estimate heritability by examining the MZ/DZ correlations. By using an ACE model individual differences in phenotypes are explained by either genetic (A), shared environment (C) or unique environment (E, including measurement error). These factors can be quantified using a twin ACE-model because monozygotic (MZ) and dizygotic (DZ) twins differ in their genetic relatedness: MZ twins share virtually 100% of their structural genome and thus have a correlation of 1 in their genetic factors, whereas DZ twins share on average 50% of their genome and thus have a correlation of .50. Shared environmental factors are events that lead to similarities between the twins and derive from e.g. family, household and residential area. Because C is the same for both twins the correlation is 1. Variance not explained by A or C results from unique environmental factors and measurement error. As E is unique for both twins, the correlation is 0. Heritability is based on the difference in genetic relatedness of MZ and DZ twins: higher MZ correlations than DZ correlations for a trait suggest genetic influences on that trait.

Replicability

Another advantage of twins is the possibility to replicate findings by creating two very similar groups: each child from a twin pair was randomly assigned to the test or replication sample. We tested our hypotheses in the test sample and replicate the findings in the replication sample. This way we optimize the chance of replication because the two samples are equal in background variables like age and gender and similar in shared environmental factors. Thus, non-replication is not easily explained by differences between the samples. Furthermore, replication of false positives and noise is unlikely, and accordingly replicated outcomes can be considered reliable. In the past years researchers have shown concerns about the lack of replication in scientific studies (see Pashler and Wagenmakers, 2012). Therefore we aimed for a replication design within our studies that examined new tasks as it enhances the validity and robustness of our findings.

Outline

The general aim of the current thesis is to gain insight in the neural correlates of fearfulness, prosocial behavior and aggressive behavior in early childhood. We focused on FA because right FA has been associated to withdrawal tendencies like in fearfulness and left FA has been associated to approach tendencies like prosocial behavior and aggressive behavior in adults and/or infants. However, there seems to be a gap in the FA-literature with regard to early childhood, even though this is an important age for the expression of temperament and social behavior. Regarding temperament we were particularly interested in the genetic and environmental influences that explain fearfulness and FA, which we examined in Chapter 2 by using behavioral genetic modeling. The genetic, shared and unique environmental influences on each of the traits are examined in univariate models. However, because both fearfulness as FA are related to withdrawal tendencies we were also interested in whether overlapping genetic or environmental influences explained individual differences in the traits, which we examined in bivariate models.

The response to social exclusion or social judgements in relation to approach tendencies and thus left FA was examined in Chapter 3 and 4. Chapter 3 focuses on prosocial behavior in reaction to social exclusion by using the “Prosocial Owl Game” (POG). To check whether prosociality during the POG was related to other, more conventional prosocial measures we related the POG results to parent-reported prosocial behavior and the donating task, a costly measurement of prosocial behavior. We also hypothesized that showing more prosocial behavior is driven by approach tendencies and thus related to left FA. The main focus of Chapter 4 is to examine aggressive behavior in response to social judgments by using the

“Social Network Aggression Task – Early Childhood” (SNAT-EC). We examined whether children react more aggressively after receiving a negative social judgment compared to a positive social judgment from a peer. We hypothesized that aggressive behavior is mediated by left FA after receiving a negative social judgment, therefore we measured FA during the task and examined state-related aggression and FA. In Chapter 5 we present the main findings and conclusions of this thesis. Furthermore, in this closing Chapter we will discuss the strengths and limitations of this thesis and directions for future research.

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CHAPTER 2

Behavioral genetics of temperament and frontal asymmetry in early childhood

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Abstract

Temperament has been suggested to be influenced by genetic and environmental factors. The current study examined genetic shared environmental and unique environmental factors accounting for variation in Fear, Effortful Control (EC), and Frontal Asymmetry (FA) in 4- to 6-year-old children using bivariate behavioral genetic modeling. We included a total of 214same-sex twin pairs: 127monozygotic (MZ) and 87 dizygotic (DZ) pairs. FA was measured during a rest electroencephalogram (EEG) recording, and Fear and EC were measured using parent report. Results show that differences between twins were best explained by genetic factors (about a quarter of the variance) and unique environmental factors (about three quarters of the variance). However, the cross-trait, within-twin correlations were not significant, implying no overlapping genetic or environmental factors on Fear and EC or on Fear and FA. Future research should try to elucidate the large role of unique environmental factors in explaining variance in these temperament-related traits.

Keywords: Temperament; frontal EEG asymmetry; behavioral genetics; early childhood

Introduction

Each child has his or her own unique temperament, which affects how the child reacts to the world. Some children will approach new situations with joy, whereas others will be more reluctant. This has a great impact on their development, and that is why temperament is one of the most widely studied features in child development (Buss & Plomin, 2014; Zentner & Shiner, 2015). Temperament has been suggested to be influenced by genetic and environmental factors form birth onward (Zentner & Shiner, 2015) and is suggested to be associated with electroencephalogram (EEG) Frontal Asymmetry (FA), the difference in activation between the left and right frontal brain areas (Rothbart, 2011). FA is related to approach and withdrawal tendencies (Harmon-Jones, Gable, & Peterson, 2010), and temperament is linked to (the modulation of) approach and withdrawal behavior (Diaz & Bell, 2012; Fox, Henderson, Marshall, Nichols, & Ghera, 2005; Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Rothbart, 2011; Smith, Diaz, Day, & Bell, 2016). Studies have shown that in particular Fear may be related to FA. More fearfulness has been related to greater right frontal activity (Fox et al., 2001; Howarth, Fettig, Curby, & Bell, 2016). In addition, studies have shown a relation between Fear and Effortful Control (EC; Cole, Zapp, Fettig, & Pérez-Edgar, 2016; Hill-Soderlund & Braungart-Rieker, 2008; Kiff, Lengua, & Bush, 2011). In the current study, we examined the associations among Fear, EC, and resting FA and explored whether temperamental features and FA are influenced by distinct or overlapping genetic and environmental factors in early childhood. Therefore, we investigated the genetic and environmental factors accounting for variation in Fear and EC, as well as in Fear and resting FA, in a sample of 4- to 6-year-old same-sex twins using bivariate behavioral genetic modeling.

The temperamental factor Fear indicates how nervous or worried a child is in relation to anticipated pain, distress, or threatening situations. Children's fearfulness can result in withdrawal behavior, for example, in a social context (Coplan, Prakash, O'Neil, & Armer, 2004; Fox et al., 2005; Henderson, Marshall, Fox, & Rubin, 2004). Infants already show fearful behavior very early in life, and experiencing fear is thought to be normal in childhood (Field & Davey, 2001; Gullone, 2000). Although the stimuli and situations that elicit fear in children change over time and both the intensity and prevalence of fear seem to decrease with age, stable individual differences in fearfulness are observed later in infancy (Gullone, 2000; Rothbart & Bates, 2006). Another important dimension of temperament is effortful control, defined as "the efficiency of executive attention, including the ability to inhibit a dominant response and/or to activate a subdominant response, to plan, and to detect errors" (Rothbart & Bates, 2006, p. 129). EC can be assessed by using parent report or behavioral measures of attentional focusing and inhibitory control (Rothbart, 2011). The development of EC starts at the end of the first year of life (Kiff, Lengua, & Zalewski, 2011), and although abilities for control continue to develop, individual differences stabilize at around 3 years of age (Kochanska, Murray, & Harlan, 2000). In support of this finding, individual differences in EC were found to be relatively stable in childhood (i.e., between 3 and 14 years of age; Tiberio et al., 2016).

Fear and EC both are part of child temperament and are suggested to be related. In addition, EC seems to play an important role in the development of emotion regulation (Rothbart & Bates, 2006) given that EC is involved in the expression of emotion. For example, in a scary or threatening situation, individuals with low EC may show high levels of fearful withdrawal behavior, whereas high EC may cause individuals to approach the situation and reduce anxiety. Studies with children have shown this association between Fear and EC; fearful infants (8–16 months old) had lower EC in early childhood (4.5–5.5 years) (Hill-Soderlund & Braungart-Rieker, 2008), social withdrawal was negatively correlated with EC in 4- to 7-year-olds (Cole et al., 2016), and Fear was negatively correlated with EC in 8- to 12-year-olds (Kiff et al., 2011). Because Fear and EC both are related to child temperament and studies have shown associations between the two traits, we tested whether the same genetic and/or environmental factors are involved in Fear and EC. We were specifically interested in estimating genetic and environmental influences in early childhood given that both Fear and EC individual differences are found to be stable from around 3 years of age (Gullone, 2000; Kochanska et al., 2000; Rothbart & Bates, 2006; Tiberio et al., 2016).

Many previous studies have used behavioral data (e.g., questionnaires, observations) to examine Fear and EC (Cole et al., 2016; Coplan et al., 2004; Fox et al., 2005; Gullone, 2000; Henderson et al., 2004; Rothbart, Ahadi, Hershey, & Fisher, 2001). However, with neurophysiological measures like FA, it is also possible to measure specific underlying behavioral tendencies (Harmon-Jones et al., 2010; Rothbart, 2011). FA is usually measured as the difference in EEG alpha power over the left and right frontal hemisphere. Research has shown that motivational tendencies are robustly related to FA; approach behavior is related to greater left than right frontal cortical activity, whereas withdrawal behavior is linked to greater right than left frontal cortical activity (Harmon-Jones & Gable, 2018; Harmon-Jones et al., 2010). Several studies have suggested relations among Fear, withdrawal, and relatively greater right FA during rest in adults (Mathersul, Williams, Hopkinson, & Kemp, 2008; Neal & Gable, 2017; Tomarken, Davidson, & Henriques, 1990), infants, and children (Fox et al., 2001; Schmidt, 2008). However, developmental samples have shown inconsistent results for the relation between Fear and FA (Diaz & Bell, 2012; Howarth et al., 2016; LoBue, Coan, Thrasher, & DeLoache, 2011). Still, FA might represent the neurophysiological mechanism underlying the withdrawn and avoidant behavior patterns resulting from Fear. A meta-analytic review indeed reported that depression and anxiety, factors that are linked to Fear, are also related to relatively greater right frontal brain activity (Thibodeau, Jorgensen, & Kim, 2006). We examined whether the same genetic and/or environmental factors are involved in Fear and FA. The direct relation between EC and FA is less well studied (cf. Kim & Bell, 2006; Smith et al., 2016). Although one study obtained a direct relation between EC and FA in children (Kim & Bell, 2006), EC might influence the relation between Fear and FA. For instance, in children with high EC, the relation between Fear and FA might be weaker because high control may enable children to overcome their fears and confront, rather than withdraw from, a scary situation. In the current study, we examined the possible modulating role of EC by computing partial correlations between Fear and FA while controlling for EC. Substantial differences between the bivariate correlations (between Fear and FA) and the partial correlations would indicate an influence of EC on the association between Fear and FA.

Behavioral genetic studies have suggested that a substantial amount of variance in Fear and EC can be explained by genetic factors (Goldsmith, Buss, & Lemery, 1997; Van Houtem et al., 2013). For example, researchers have estimated that genetic factors accounted for 74% and unique environmental factors for 26% of variance in individual differences in parent-reported Fear in 8-year-old children (Clifford, Lemery-Chalfant, & Goldsmith, 2015). Regarding EC, one study indicated that dominant genetic factors accounted for 68% and unique environmental factors for 32% of variance in parent-reported EC in 8-year-olds (Lemery-Chalfant, Doelger, & Goldsmith, 2008).

Only a few studies have investigated the behavioral genetics of FA. In female adult participants, genetic factors were estimated to account for 27% of the variance and unique environmental factors for 73% of the variance in FA (Anokhin, Heath, & Myers, 2006). A study in young adults found that genetic factors accounted for 32% (in men) and 37% (in women) of individual differences in FA (Smit, Posthuma, Boomsma, & De Geus, 2007). Moreover, Smit et al. (2007) examined the relation between FA and risk for anxiety and depression (also related to fearfulness as described above) in a bivariate genetic analysis. They found that FA and the risk for anxiety/depression correlated significantly only in a subsample of young women and concluded that the correlation was explained by overlap in genetic factors. The influence of genetic shared and unique environmental factors on characteristics such as Fear, EC, and FA, however, changes over the lifespan (Briley & Tucker-Drob, 2014; Kandler & Papendick, 2017; Scaini, Belotti, & Ogliari, 2014), and research in young children is lacking. Conducting behavioral genetic research with a focus on developmental populations will, therefore, add important information to the current literature.

We conducted a twin study including 4- to 6-year-old same-sex twins to examine the behavioral genetics of Fear, EC, and FA using bivariate behavioral genetic modeling. Because previous research has suggested associations between Fear and EC and between Fear and FA (Cole et al., 2016; Fox et al., 2001; Hill-Soderlund & Braungart-Rieker, 2008; Howarth et al., 2016; Kiff et al., 2011; Rothbart, 2011; Schmidt, 2008), we were interested in the extent to which the same and/or different genetic and environmental factors account for variation in these temperamental characteristics.

Method

Participants

Participants took part in a larger longitudinal intervention study of the Leiden Consortium on Individual Development (L-CID; Euser et al., 2016). We recruited families with same-sex twins born between 2010 and 2013 via municipal authorities in the western part of The Netherlands. Children with disabilities or neurological impairments that prevented them from completing the tasks were excluded (i.e., congenital disability, psychological disorder, chronic illness, hereditary disease, or visual or hearing impairment). For the current study, we used data from the second wave of the data collection in which 215 twin pairs participated. We asked both parents from each family to take part in the study. The primary parent (i.e., the parent who spends the most time with the children) was invited for each visit and asked to complete a set of questionnaires; in most cases (94%), the primary parent was the biological mother of the twins. The other parent was asked to complete questionnaires as well.

One twin pair was excluded from the analyses because of missing data on all variables used in the current study. Another 12 participants had missing data for Fear and EC because both parents did not complete the Child Behavior Questionnaire (CBQ; Rothbart et al., 2001). There was also missing data on FA (in total 102 incomplete or missing twin pairs) because of insufficient artifact-free EEG data (n = 50), technical problems during EEG acquisition (n = 29), or refusal to wear the EEG net (n = 61). All participants (also with partially missing data) could be included in the behavioral genetic analysis because it employs full information maximum likelihood (FIML) modeling, which can deal with missing data. FIML estimates a likelihood function for each individual in the dataset based on all variables with valid data (Enders, 2001). The final sample, therefore, consisted of 214 twin pairs, 127 of which were monozygotic (MZ) and 87 of which were dizygotic (DZ). Zygosity was determined by analyses of DNA samples collected by buccal swabs. When the DNA samples were missing, zygosity was based on the zygosity questionnaire (Rietveld et al., 2000), which was filled out by the primary parent. The mean age of the MZ twins was 4.82 years (SD = 0.61, confidence interval (CI) [3.86–6.54]) and of the DZ twins was 4.70 years (SD = 0.53, CI [3.93–6.14]). See Table 1 for participant characteristics.

Both parents provided written informed consent at the start of the study, and study procedures were approved by the local ethics committee and the Central Committee on Research Involving Human Subjects in The Netherlands (No. NL49069.000.14, "Samen Uniek"). Participating families received financial reimbursement after each visit, and the children received a small gift.

Table 1. Participant characteristics

	MZ twins	DZ twins
N (total twin pairs)	127	87
Girls (%)	51%	53%
Mean age in years (SD)	4.82 (.61)	4.70 (.53)
Age range	3.86 – 6.54	3.93 – 6.14

Procedure

Families were invited to the lab at Leiden University. One week before the lab visit, the parents received an e-mail asking them to complete several online questionnaires, including the CBQ (Rothbart et al., 2001). During the lab visit, each co-twin was randomly assigned to one of two order conditions (starting with a block of behavioral tasks or EEG measures) and to a research assistant who supervised the tasks and motivated the child throughout the test session. One block of tasks consisted of EEG measures, including a baseline and task EEG measurements. The other block included several behavioral tasks and parent–child interaction tasks (results reported elsewhere). After completing the first block of tasks, the participants switched rooms and completed the other block of tasks. The total duration of the lab visit was approximately 3 h. At the start of the block including the EEG measurement, the procedure was explained to the parent and child by the experimenter. Next, the child was fitted with the electrode net. The first measurement was a 3-min resting baseline EEG measurement (see below). Next, a task lasting approximately 15 min was conducted (results presented elsewhere; van Wijk et al., 2017).

Measures and data processing

Child behavior questionnaire. To measure child temperament, parents completed the subscales Fear (12 items), Attentional Focusing (short form, 6 items), and Inhibitory Control (short form, 6 items) of the CBQ for each cotwin separately. Together, the subscales Attentional Focusing and Inhibitory Control form the dimension Effortful Control (EC). Items were rated on a 7-point Likert scale ranging from extremely untrue for your child (1) to extremely true for your child (7). When the behavior described in the item was not previously observed in the child, it was rated as not applicable (8). These items were coded as missing values and were not included in subscale scores. Previous studies have shown acceptable internal consistency of the subscales: Fear a = .70, Attentional Focusing a = .75, and Inhibitory Control a = .72 (Putnam & Rothbart, 2006; Rothbart et al., 2001).

To limit the number of questions for the parent, we used planned random missing items in the CBQ (Graham, Taylor, Olchowski, & Cumsille, 2006; Little & Rhemtulla, 2013). For both the subscale Fear and the dimension EC, 3 items were always included, and of the remaining 9 items, 6 items were randomly selected to be included for each co-twin. Missing value analyses confirmed that data were missing completely at random (MCAR); p values for Little's MCAR test (Little & Rubin, 1989) ranged between .18 and .77. We used multiple imputation (Rubin, 1987; Schafer & Olsen, 1998) in SPSS 23 (IBM, Armonk, NY, USA) to handle missing items. A total of 100 imputed datasets were generated for each subscale, for each parent, and for each child separately (the oldest and youngest co-twins within families were randomly assigned to Twin Group A or Twin Group B). The average Cronbach's alpha for the imputed data of the primary parent was M = .73 for Fear and M = .82 for EC. For the other parent, the average Cronbach's alpha of the imputed data was M = .64 for Fear and M = .82 for EC. Total scores were then computed for Fear and EC for each dataset, and the datasets were pooled and merged. The pooled total scores for Fear and EC were used in subsequent analyses.

The correlations for Fear and EC between the pooled scores from the primary parent and the other parent were substantial and significant (Fear: Child 1 r = .47 and Child 2 r = .52; EC: Child 1 r = .51 and Child 2 r = .52, all ps < .01). Because a paired-samples t test showed one significant difference between the primary parent and the other parent on EC Child 2, t(157) = 3.07, p < .01, we used the standardized values to compute a mean score based on both parents' ratings on Fear and EC, which we used in further analyses. When one of the parents did not fill out the CBQ (n = 17 for the primary parent and n = 45 for the other parent), the score of the parent who did fill out the CBQ was used in further analyses (which is taken into account by computing the mean score). Both Fear and EC were normally distributed (z skewness and z kurtosis values did not exceed ±3), and there were no outliers (all |z| < 3.29).

Frontal EEG asymmetry. EEG was recorded during a 3-min resting baseline. The child was instructed to alternatingly open or close his or her eyes for 30 s each (3x30 s eyes open and 3x30 s eyes closed). The computer played an audio message telling the child to close his or her eyes and displayed a drawing of closed eyes when the child needed to close the eyes. After 30 s, an audio message was played saying that the child could open his or her eyes again. During the eyes open trials, the child saw a color-changing dot on the screen to focus attention and avoid excessive eye movements.

A 64-channel HydroCel Geodesic Sensor Net and NetStation software (Electrical Geodesics, Eugene, OR, USA) with a NetAmps300 amplifier were used to record the EEG. To ensure a good signal, each electrode was adjusted to keep impedances below 100 kO. To avoid fatigue, irritability, and loss of attention in young children, we minimized preparation time by adjusting and collecting data from only a subset of the electrodes (number in brackets): F3

[12], F4 [60], F7 [18], F8 [8], C3 [20], C4 [50], T7 [24], T8 [52], P3 [28], P4 [42], P7 [30], P8 [44], left [29] and right [47] mastoids, and two electrodes [62, 63] placed directly below the eyes. During recording, the reference was Cz and data were low-pass filtered at the Nyquist frequency (i.e., 100 Hz) for the sampling rate of 250 Hz. After applying a 0.3-Hz high-pass filter (99.9% pass-band gain, 0.1% stop-band gain, 1.5 Hz roll-off), data were exported for further processing using Brain Vision Analyzer (BVA) 2.0 software (Brain Products GmBH, Gilching, Germany). The EEG was low-pass filtered at 30 Hz (-3 dB, 48 dB/octave) and Cz was used as reference. The six 30-s trials were segmented into 2-s segments with 1-s overlap. Segments containing artifacts (i.e., segments in which the difference between the largest and smallest values was larger than 200 lV or in which the difference between the largest and smallest values within any 100-ms interval was smaller than 0.5 lV in any channel) were removed, and bad channels were deleted from an individual dataset if the channel contained artifacts in more than 50% of segments. A fast Fourier transformation (0.5 Hz resolution, 100% Hamming window) was used to compute power values (lV2). Power values were averaged per condition over the artifact-free segments. The minimum requirement for a child’s data to be included in further analyses was 28 segments per condition (equal to 56 s over the two conditions). On average, 63 segments per condition were included (eyes closed: M = 61, CI [29–87]; eyes open: M = 65, CI [29–87]).

Power values were then averaged across the frequency range of 6–10 Hz (alpha power in young children; Marshall, Bar-Haim, & Fox, 2002) to obtain alpha power for each condition. With a natural log transformation, the data distributions were normalized. Based on other studies of FA (for a review, see Coan & Allen, 2004) and studies that specifically investigated the contribution of genetic and environmental factors to FA (Anokhin et al., 2006; Smit et al., 2007), we used electrodes F4 and F3 to compute FA. Other electrode sites were not analyzed. Alpha activity over left frontal areas (electrode F3) was subtracted from alpha activity over right frontal areas (electrode F4) to compute FA. The data showed four outliers ($|z| > 3.29$) that were winsorized (Tabachnick & Fidell, 2006). To check the reliability of our FA measure, we computed split half reliability; FA was computed separately for odd and even segments (following the same procedures as described above), and intraclass correlations between measures for odd and even segments were computed. Results showed high intraclass correlation coefficients (condition eyes open: Child 1 $r = .86$, $p < .01$ and Child 2 $r = .90$, $p < .01$; condition eyes closed: Child 1 $r = .88$, $p < .01$ and Child 2 $r = .90$, $p < .01$), indicating that the measurement was reliable and did not show much variance. Furthermore, the correlation between FA in the two conditions (eyes open and eyes closed) was high ($r = .87$, $p < .001$). Therefore, we decided to average across the two conditions to obtain one value of FA per child, which we used in all subsequent analyses.

There were 42 children with sufficient artifact-free EEG data for one condition only (eyes open [$n = 33$] or eyes closed [$n = 9$]). To enhance the number of twin pairs included in our study and because of the high correlation between the eyes open and eyes closed conditions ($r = .88$, $p < .01$), we estimated the value of the missing condition based on the value of the other condition using the regression equation obtained in the subsample of children with sufficient data for both conditions ($n = 246$). Using this method, data of 22 twin-pairs could be imputed and included in the bivariate behavioral genetic modeling analyses.

Data analyses

Individual differences in phenotype can be accounted for by genetic (A), shared environmental (C), and unique environmental (E; also includes measurement error) factors. These factors can be quantified using a twin ACE model because MZ and DZ twins differ in their genetic relatedness; MZ twins share virtually 100% of their structural genome and, thus, have a correlation of 1 in their genetic factors, whereas DZ twins share on average 50% of their genome and, thus, have a correlation of .50. Shared environmental factors are events that lead to similarities between the twins and derive from family, household, residential area, and the like. Because C is the same for both twins, the correlation is 1. Variance not explained by A or C results from unique environmental factors and measurement error. Because E is unique for both twins, the correlation is 0. We computed twin correlations for Fear and EC to examine whether the within-trait, cross-twin correlations were larger for MZ twins as compared with DZ twins because this would suggest heritability. In addition, we computed partial twin correlations for Fear and FA with EC as a covariate. In a bivariate twin model, the contribution of A, C, and E factors to the variance in Fear, EC, and FA was examined. In addition, the contribution of A, C, and E to the association between Fear and EC, as well as between Fear and FA, was examined.

Bivariate behavioral genetic analyses were performed with Open Mx (Version 2.7.4) in R (Version 3.3.2) using structural equation modeling. We first used a saturated Cholesky decomposition model to compare with the full bivariate ACE model. We then further tested the ACE model against CE, AE, and E bivariate models, selecting the model with the best goodness of fit. This fit is operationalized as the -2 log likelihood statistic, which is distributed as chi-square (χ2). The χ2 test represents the difference in log likelihood between two nested models, with df (degrees of freedom) being the difference in df between the models. When χ2 is less than 3.84 and shows a p value greater than .05, the more parsimonious model (with fewer parameters) does not significantly deteriorate the fit and, therefore, is preferred. Furthermore, to compare model fit between non-nested models (AE and CE), we used Akaike's information criterion (AIC); better fit is indicated by a lower AIC value. For the model with the best fit, we computed the path loadings. To quantify the relative influence of each of the factors, we first standardized and then squared the path loadings. The correlation within a twin between two traits is represented by the cross-trait, within-twin correlations. When the cross-trait, within-twin correlation was significant, we calculated the extent to which the same genetic or environmental factors influenced both Fear and EC or both Fear and FA, based on the correlations and the standardized path loadings (see Treur, Boomsma, Ligthart, Willemsen, & Vink, 2016).

Results

Twin correlations

Descriptive statistics are summarized in Table 2. A correlation matrix split by zygosity is shown to examine whether the within-trait, cross-twin correlations were larger for MZ twins as compared with DZ twins because this would suggest genetic influence (see Table 2). MZ twins indeed showed higher correlations than DZ twins for Fear and EC but not for FA (Fear: $r_{MZ} = .39$, $p < .01$ and $r_{DZ} = -.06$, $p = .58$; EC: $r_{MZ} = .38$, $p < .01$ and $r_{DZ} = -.26$, $p < .05$; FA: $r_{MZ} = .17$, $p = .16$ and $r_{DZ} = .25$, $p = .12$). The between-trait correlations were significant in MZ twins for EC and FA in Child 1 ($r = -.21$, $p < .05$) but not in Child 2. This means that only in Child 1 of MZ twins is more effortful control related to relatively greater left frontal brain activity. In DZ twins, the correlations between Fear and FA in Child 1 ($r = .30$, $p < .05$) and between Fear in Child 1 and FA in Child 2 ($r = -.29$, $p < .05$) were significant. No other significant correlations were found. Partial twin correlations between Fear and FA, corrected for EC (see Table 2) showed that the correlation between Fear and FA in Child 1 in DZ twins was significant ($r = .36$, $p < .05$). The correlation between Child 1 Fear and Child 2 FA in DZ twins was not significant anymore ($r = -.17$, $p = .31$). No other significant correlations were found between Fear and FA. Therefore, we concluded that EC does not have a large influence on the relation between Fear and FA.

Bivariate ACE model fitting and path loadings

We used two bivariate ACE models to estimate the influence of genetic and shared and unique environmental factors on Fear and EC as well as on Fear and FA. The results of the bivariate models are shown in Table 3. Standardized squared path loadings of each best fitting bivariate model are displayed in Fig. 1. The percentages of A, C, and E explaining variation in Fear may be slightly different among the models because these depend on the specific combination of traits. As a robustness check, univariate models for Fear, FA, and EC are described in the Appendix A. The cross-trait, within-twin correlation was not significant in any of the bivariate models (Fear and EC: r = -.05; Fear and FA: r = .06); thus, no meaningful analyses of the influence of A, C, or E factors on the overlap between the traits could be performed.

Table 2. Correlations between Fear, EC and FA within and across traits and twins

	Fear C1	Fear C2	EC C1	EC C2	FA C1	FA C2	n	*M*	*SD*
Fear C1	-	-.06	-.16	-.05	**.30***	**-.29***	86	.11	.98
Fear C2	**.39****	-	.01	-.06	-.20	-.04	86	.18	.97
EC C1	-.04	.13	-	**-.26***	-.07	-.12	86	.01	.93
EC C2	-.05	.02	**.38****	-	.08	.09	86	.01	.91
FA C1	.07	-.02	**-.21***	-.13	-	.25	50	-.06	.26
FA C2	-.08	-.01	-.01	-.07	.17	-	58	-.13	.27
n	122	122	122	122	92	88			
M	-.06	-.08	.00	-.06	-.11	-.10			
SD	.84	.82	.88	.88	.23	.22			
Partial correlations between Fear and FA, controlled for EC									
Fear C1					**.36***	-.17			
Fear C2					-.28	-.07			
FA C1	-.01	-.04							
FA C2	-.16	.01							

Note. MZ twins below the diagonal, DZ twins above the diagonal. Sample size, means and standards deviations for MZ twins are presented in the horizontal rows and for DZ twin in vertical rows. Means for Fear and EC are standardized.

*p < .05; ** p < .01

Table 3. Bivariate model fitting of Fear and EC and Fear and FA

	Model	Estimated variables	-2LL	AIC	Compared with	$\Delta \chi^2$	Δ *df*	*P*
Fear and EC	Saturated model	28	2110.02	502.02	-	-		-
	Full ACE model	11	2149.60	507.60	1	39.58	17	0.001
	AE model	**8**	**2149.60**	**501.60**	**2**	**0.000**	**3**	**1.000**
	CE model	8	2164.04	516.04	2	14.44	3	0.002
	E model	5	2173.42	519.42	3	23.82	3	0.001
Fear and FA	Saturated model	28	1033.74	-318.26	-	-		-
	Full ACE model	11	1065.33	-320.67	1	31.59	17	0.017
	AE model	**8**	**1067.32**	**-324.68**	**2**	**1.99**	**3**	**0.575**
	CE model	8	1071.28	-320.72	2	5.95	3	0.114
	E model	5	1090.48	-307.52	3	25.15	3	0.000

Note: The best fit for each bivariate model is shown in bold. -2LL, difference in likelihood ratio test between the two compared models; AIC, Akaike's information criterion, to compare model fit between the non-nested models AE and CE, a lower value indicates a better fit; Δ χ2, delta chi square, represents the difference in log likelihood between the two compared models using a chi square distribution; Δ df, difference in degrees of freedom between the two compared models

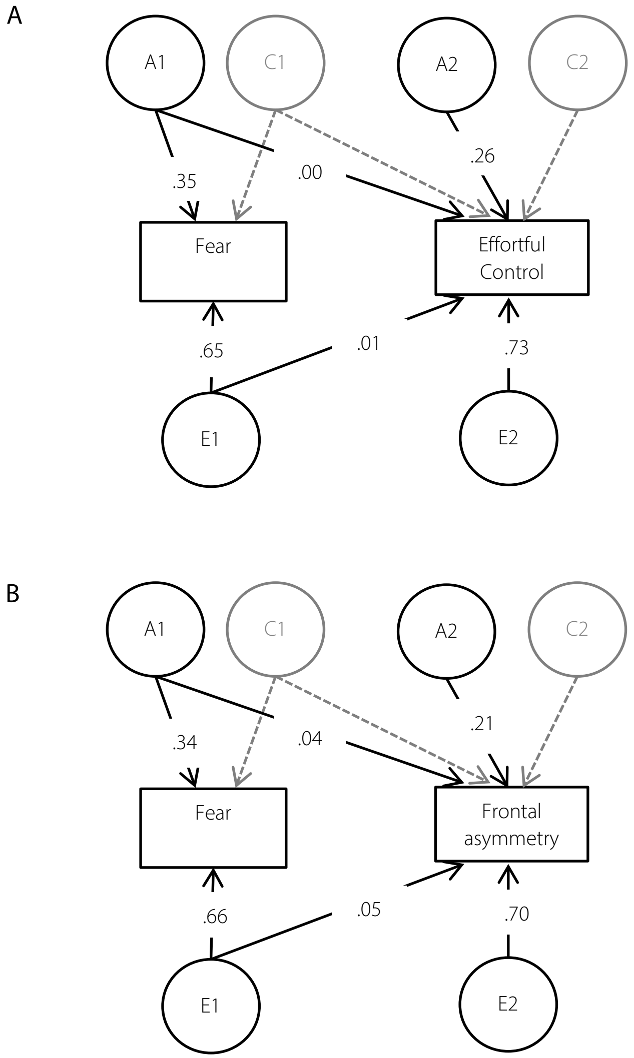

Figure 1. Bivariate twin models with squared path loadings. (A) Fear and EC: AE model, and (B) Fear and FA: AE model. In each model the first factor is explained by the path loadings of A1, C1 and E1 and the second factor is explained by the sum of A1 and A2, C1 and C2 or E1 and E2. Results are shown for the best fitting model, greyed out factors and path loadings were not included.

Fear and EC. The results of the bivariate model with Fear and EC showed that the AE model had the best fit ($\Delta\chi^2 < 3.84$, $p > .05$), indicating that genetic and unique environmental factors account for the variation in Fear and EC. Path loadings of the model (see Fig. 1A) show that individual differences in Fear were explained by genetic factors (35%) and unique environmental factors (65%). Variation in EC was explained by genetic factors (26%) and unique environmental factors (74%).

Fear and FA. The Fear and FA combination also shows that the AE model had the best fit ($\Delta\chi^2 < 3.84$, $p > .05$), indicating that genetic and unique environmental factors account for the variation in Fear and FA. The path loadings (see Fig. 1B) show that individual differences in Fear were explained by genetic factors (34%) and unique environmental factors (66%). Variation in FA was explained by genetic factors (25%) and unique environmental factors (75%).

Discussion

The current study investigated genetic and environmental factors accounting for variation in temperamental traits. We specifically focused on the relation between Fear and EC, as well as between Fear and the possible neural correlate FA, in bivariate behavioral genetic models. Results showed that individual differences in parent-reported Fear and EC, as well as children's FA, were best explained by genetic factors (for about one quarter) and by unique environmental factors (for about three quarters). Cross-trait, within-twin correlations were not significant in any model, precluding overlapping genetic or environmental factors on Fear and EC or on Fear and FA.

In line with previous studies (Anokhin et al., 2006; Clifford et al., 2015; Goldsmith et al., 1997; Lemery-Chalfant et al., 2008; Smit et al., 2007; Van Houtem et al., 2013), we found that Fear and EC and Fear and FA were best explained by genetic and unique environmental factors (AE models). Still, most of the variation between individuals was explained by unique environmental factors. Research conducted with 8-year-old children found a larger influence of A to explain individual differences in reported Fear and EC (Clifford et al., 2015; Lemery-Chalfant et al., 2008). The twins in our study were on average 3 years younger, so the difference in the ratio of A and E might result from developmental changes. Indeed, a meta-analysis by Kandler and Papendick (2017) showed that the relative contribution of A and E to personality traits changes over the lifespan. However, their results suggest that the influence of genetic factors on personality stability slightly decreases with age, whereas the influence of unique environmental factors increases. Longitudinal studies of the behavioral genetics of Fear, EC, and FA across different age groups are necessary to draw firm conclusions about increases or decreases in A and E.

A potential unique environmental factor influencing Fear, EC, and FA is parenting. Child temperament may elicit certain parenting behaviors, which in turn enhance specific temperamental characteristics (see review in Kiff et al., 2011). With regard to Fear in particular, one study suggested that parental practices such as warmth–reasoning and harshness–hostility are unique environmental factors that influence anxiety in 10- to 18-year-old children (Chen, Yu, & Zhang, 2016). Regarding FA, children who received low-quality maternal caregiving behavior showed relatively greater right FA and more social inhibition at 3 years of age (Hane, Henderson, Reeb-Sutherland, & Fox, 2010). Parenting is often assumed to be a shared environmental factor (i.e. a factor that leads to similarities between the twins), but it can also be a unique environmental factor. For example, although maternal sensitivity is mainly a shared environmental factor influencing infant attachment, attachment security of one twin was also uniquely affected by the relation of the parent with the other twin (Fearon et al., 2006). In addition, twins report that they perceive different parenting (Hannigan, McAdams, Plomin, & Eley, 2016). This indicates that parenting varies between co-twins and may lead to differences between children; as a result, parenting is at least partly a unique environmental factor.

It is important to note that the E factor includes not only unique environmental factors but also measurement error. Recently, a longitudinal cross-cultural study investigating parenting and behavioral and emotional adjustment (based on self-reports) in children (8, 10, and 12 years old) showed that most variation was explained by within-person variability rather than between-person or between-group variability (Deater-Deckard et al., 2018). Accordingly, we believe that measurement error always plays a role when collecting data. However, to minimize measurement error, we used a well-validated instrument that is often used to measure temperament in young children, the CBQ (Putnam & Rothbart, 2006; Rothbart et al., 2001). Our data showed acceptable internal consistency for the subscales Fear and EC. Regarding FA, we observed excellent split-half reliability, suggesting that measurement error is not a factor of great concern. However, some uncertainty regarding the most appropriate quantification of FA in young children remains (see, e.g., Peltola et al., 2014) despite reasonable arguments for the comparability of 6- to 10-Hz activity in young children with adult alpha (Marshall, Bar-Haim, & Fox, 2002).

In addition, the question may arise as to what extent FA reflects a stable trait. In fact, a single measure of resting FA probably reflects a mixture of trait- and state-related variance. Hagemann, Naumann, Thayer, and Bartussek (2002) suggested that 40% of the variance is due to state-related fluctuations (reflecting the participant's response to the recording situation) and 60% is stable trait variance. If genetic factors influence mostly traits, the maximum genetic influence on individual differences in FA can never exceed 60% (Smit et al., 2007) and the presence of state-related variance may help to explain the low A and large E components we obtained. On the other hand, it is possible that MZ twins react more similarly to specific situations, including the laboratory environment and EEG measurement. In that case, not only is the stable trait variance shared between MZ twins but also the state variance should be more similar between MZ twins than between DZ twins. More research is necessary to determine the maximum influence of genetics on FA when using twin models.

With regard to the association between Fear and EC or between Fear and FA, our results showed only few significant cross-trait correlations. Moreover, the bivariate models did not find any significant cross-trait, within-twin correlations, suggesting that the traits were not associated. This is not in line with our hypotheses and previous studies reporting associations between these traits in young children (Cole et al., 2016; Fox et al., 2001; Hill-Soderlund & Braungart-Rieker, 2008; Howarth et al., 2016; Kiff et al., 2011; Rothbart, 2011; Schmidt, 2008). In addition, partial correlations between Fear and FA while controlling for EC were only slightly different from the correlations between Fear and FA without controlling for EC, suggesting that EC did not affect the relation between Fear and FA. One explanation for the lack of associations between the constructs in the current study is that we used trait-related measures rather than settings evoking specific behaviors (such as fearful behavior and right FA during a fear-inducing task). We obtained overall ratings of Fear and EC from parents and FA during a resting EEG measurement because we were specifically interested in individual differences in more stable, task-independent traits. Indeed, other studies using parent-reported Fear and children's resting FA have also failed to find significant relations (Diaz & Bell, 2012; LoBue et al., 2011) or suggest more complex relationships. Howarth et al. (2016), for example, did not find a relation between Fear and FA in 10-month-olds, but they found that parent-reported Fear in 36-montholds predicted right FA when the children were 48 months old. Another possibility is that FA acts like a moderator of temperamental characteristics (cf. Coan & Allen, 2004).

Our study has some limitations that should be addressed in future research. First, about 33% of the children provided no usable FA data (n = 140). However, obtaining EEG measures from young children is challenging, and 40% is a common attrition rate (Bell & Cuevas, 2012). Moreover, the missing FA data is not of great concern for the current study because the behavioral genetic analyses uses FIML modeling that is robust to missing data. Still, sample size remains an important issue. Future studies should aim at including larger samples, for example, by combining studies from several research groups. Second, because of developmental differences and issues relating to the assessment and quantification of both behavioral and neural indices in 4- to 6-year-olds (including quality and quantity of data and the selection of EEG frequency bands), our results cannot be directly compared with adult studies. Future research should investigate developmental patterns of temperament and FA using measures obtained at several time points from the same individuals. With regard to the reliability of FA, we suggest that future research should determine the optimal number of segments needed to ensure good quality and quantity of EEG measures used for FA computation (see also van Wijk et al., 2017). Third, the generalizability of findings from twin research to singletons is sometimes questioned. However, research has shown that singletons and twins do not differ on temperament (Goldsmith & Campos, 1990) or personality (Johnson, Krueger, Bouchard, & McGue, 2002); therefore, we assume that the individual differences in temperament in early childhood are generalizable from twins to singletons. On the other hand, parents of MZ twins might find it more difficult than parents of DZ twins or singletons to indicate the differences between their children on a temperament questionnaire. This could lead to an overestimation of genetic factors. To overcome this problem, in future studies co-twins could report on their own temperament and on their sibling's temperament, especially in studies with older children. It should be noted that we used Fear and EC ratings from both the primary parent and the other parent to decrease the influence of reporter bias.

In sum, our findings indicate that individual differences in young children's temperament-related traits are best explained by a combination of genetic factors and unique environmental factors. Unique environmental factors in particular accounted for a large proportion of the variance. Exactly which environmental factors are important for temperament development is an important topic for future research given that child temperament is a predictor for success later in life (Zentner & Shiner, 2015). Gaining insight into the specific environmental factors that contribute to temperament will ultimately facilitate support for children who cope with fearfulness or other difficulties with emotion regulation.

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CHAPTER 3

Prosocial Owl Game: Assessing Compensation for Social Exclusion in Early Childhood

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Submitted

Abstract

This study examined prosocial reactions to social exclusion in 4–6 year old children with a newly developed task: the Prosocial Owl Game (POG). In the POG, two cartoon owls exclude a third owl, and the child can compensate for this exclusion by giving the excluded owl the next turn. A replication design with two samples (both n = 214) consistently showed that the vast majority compensated for social exclusion in the first trials and that individual differences arise when the game progresses. Individual differences in the POG could not be explained by frontal asymmetry, parent-reported prosociality or donating behavior. However, substantial heritability estimates indicated that variance in the POG cannot be explained only by measurement error. The POG is a promising measure of prosocial compensating behavior in early childhood, but environmental influences on variation in POG performance need further investigation.

Keywords: Prosocial Owl Game, prosocial behavior, social exclusion, early childhood, frontal asymmetry, EEG

Introduction

On the playground children approach each other and invite their peers to play along. This type of behavior is considered prosocial behavior, or "any action that serves to benefit another person" (Schroeder & Graziano, 2015), in particular when the peer has previously been excluded from a game. Prosocial behavior may also have positive consequences for the actor in terms of social outcomes such as peer acceptance (Sebanc, 2000; Layous, Nelson, Oberle, Schonert-Reichl, & Lyubomirsky, 2012), mental health (Schwartz, Meisenhelder, Ma, & Reed, 2003), life-satisfaction, and academic achievement (Caprara, Barbaranelli, Pastorelli, Bandura, & Zimbardo, 2000; Caprara & Steca, 2005). In addition, experiencing social exclusion has negative consequences and is related to feelings of pain (Eisenberger & Lieberman, 2005) and aggressive behavior (Twenge, Baumeister, Tice, & Stucke, 2001). Prosocial individuals who are aware of the negative consequences of social exclusion for peers may be inclined to actively include an excluded peer and thereby compensate for social exclusion by others. Even though compensating behavior can be observed in children, studies objectively examining this specific kind of prosocial behavior in early childhood are lacking. In the current study we present a new task, the "Prosocial Owl Game" (POG), to measure prosocial compensating behavior in early childhood. The task is based on the Prosocial Cyberball Game, which has been used in older children, adolescents, and adults (Riem, Bakermans-Kranenburg, Huffmeijer, & van IJzendoorn, 2013; Vrijhof et al., 2016; Van der Meulen, van IJzendoorn, & Crone, 2016; Van der Meulen et al., 2017).

The development of prosocial behavior starts early in life, as even infants show helping or sharing behavior (Paulus, 2014). Factors that play an important role in the development of prosocial behavior are social-cognitive skills, differentiation between self and others, empathy, and moral reasoning (Paulus, 2014; Eisenberg, Spinrad, & Knafo-Noam, 2015). In general, prosocial behavior seems to increase from infancy to adolescence (Eisenberg et al., 2015). However, from the age of three children become more selective to whom they are prosocial based on friendships, gender and social rules (Hay & Cook, 2007). Individual differences in prosocial behavior may be associated with child temperament and environmental factors (e.g.

whether prosocial behavior is being probed or not). However, studies showed inconsistent findings concerning the relation between prosocial behavior and child temperament or environmental factors due to differences in context and type of prosocial behavior measured (Eisenberg et al., 2015). Thus far, the field has been unable to identify factors reliably characterizing children who show more prosocial behavior than others (Thompson & Newton, 2013; Eisenberg et al., 2015). Although the influence of situational factors, like probing or modelling, may contribute to the inconsistency of findings (Van IJzendoorn, Bakermans-Kranenburg, Pannebakker, & Out, 2010; Wildeboer et al., 2017), the lack of standard measurement tools may also be accountable for this state of affairs.

Prosocial behavior in early childhood is often assessed using questionnaires (e.g. parent or teacher reports) or in a variety of observational settings (e.g. helping, sharing or comforting; Paulus, 2018). Because parents and teachers may give socially desirable answers and are not constantly in the child's presence, reported prosocial behavior might not always converge with observed prosocial behavior (Wildeboer et al., 2017). Observations of helping, sharing, and comforting behaviors may be less biased, but are time consuming. Moreover, they are found to be only modestly related, probably because different tasks require different social-cognitive skills and motivations (Dunfield & Kuhlmeier, 2013). Furthermore, in the literature a distinction is made between costly and non-costly prosocial behavior. Prosocial behavior can be costly when the participant has to give up a possession, for instance in sharing tasks (i.e. money or stickers). Whether a child is inclined to share, and thus to show costly prosocial behavior, is influenced by the recipient's needs as well as the resource costs and the benefits for the participant (Martin & Olson, 2015). Prosocial behavior is non-costly in situations where the participant is helping or comforting without giving up any of his/her own possessions. Because of the limitations of existing measures, we developed an early-childhood version of an objective measure of non-costly prosocial behavior, enabling the examination of the development of prosocial behavior over time.

The Prosocial Cyberball Game (PCG; Riem et al., 2013) was developed to examine prosocial behavior in response to social exclusion. The task is based on the Cyberball Game, a virtual ball-tossing game with three players where, at a certain point in the game, two players no longer toss the ball to an excluded player (Williams & Jarvis, 2006). The PCG was adapted to a four-player game, including the participant and three unknown others. During the PCG the participants themselves were not excluded but they could choose to toss the ball to the player that was excluded by the two other players. Several studies have shown that from the age of seven onwards, individuals behave prosocially towards the excluded player by showing compensating behavior (i.e., tossing more than a third of their throws to the excluded player; Riem et al., 2013; Vrijhof et al., 2016; Van der Meulen et al., 2016, 2017). In adults, fMRI results showed increased activation in the temporal parietal junction, an area related to social reasoning and empathy (Decety & Lamm, 2006), and the nucleus accumbens, an area related to experiencing rewards (Lieberman & Eisenberger, 2009), during PCG compensating behavior (Van der Meulen et al., 2016). In 7-10-year-old children the posterior cingulate cortex/precuneus, an area related to empathy and mentalizing (Hyatt, Calhoun, Pearlson, & Assaf, 2015), was associated with prosocial compensating behavior. This suggests that social brain network areas related to empathy, rewards and mentalizing, are involved in compensating behavior during social exclusion.

The neural correlates of compensating behavior in early childhood have not been examined yet. We examined whether frontal asymmetry (FA), the difference between left and right frontal brain activity as measured with electroencephalography (EEG), is related to prosocial compensating behavior during social exclusion. According to the motivational direction model, FA is related to approach and withdrawal tendencies: relatively greater left activity reflects approach motivation and behavior whereas relatively greater right activity reflects withdrawal motivation and behavior (Harmon-Jones, Gable, & Peterson, 2010; Harmon-Jones & Gable, 2018). Showing prosocial behavior, for example by compensating for social exclusion, reflects a tendency to confront (rather than withdraw from) a situation and may be considered approach behavior toward the targeted individual. One study with infants (14-, 18- and 24-month-olds) indeed showed that greater left frontal activity was related to prosocial behavior in the form of understanding distress and global empathy for the mother in a behavioral comforting task (Paulus, Kühn-Popp, Licata, Sodian, & Meinhardt, 2013). Also, greater left frontal activity in adults was related to larger donations to charity, a form of costly prosocial behavior (Huffmeijer, Alink, Tops, Bakermans-Kranenburg, & van IJzendoorn, 2012).

Compensating behavior in reaction to social exclusion has thus far only been investigated in children of at least 7 years old, adolescents, and adults (Riem et al., 2013; Vrijhof et al., 2016; Van der Meulen et al., 2016, 2017). Knowledge about prosocial reactions to social exclusion on a behavioral and neural level in early childhood (4-6-year-olds) is still lacking. The current study examined this specific kind of prosocial behavior in early childhood by using a newly developed task, the "Prosocial Owl Game" (POG). We hypothesized that young children already notice social exclusion during a virtual game and can react prosocially by compensating for the exclusion. To check whether compensating behavior was related to other more conventional measures of prosocial behavior in early childhood we correlated the outcome of the POG with parental reports on social development and a donating task (observed costly prosocial behavior). However, because prosocial behavior is a multidimensional construct (Paulus, 2018; Padilla-Walker & Carlo, 2015), we had no strong expectations about these associations. Finally, we expected that greater left frontal activity at rest would be related to more prosocial compensating behavior, as we hypothesize that prosocial behavior is related to approach motivation. To validate the POG and replicate the findings within the current study, we used a twin sample to create two samples, a test and replication sample. This way we optimized the chance of replication because the two samples are equal in background variables like age and gender and similar in shared environmental factors. Thus, non-replication is not easily explained by differences between the samples. Furthermore, replication of false positives and noise is unlikely, and accordingly replicated outcomes are optimally reliable.

Methods

Participants

The participants in this study took part in the larger experimental longitudinal twin study of the Leiden Consortium on Individual Development (L-CID, Euser et al., 2016). Via municipal authorities in the western part of the Netherlands we recruited families with same-sex twins born between 2010 and 2013. Twins and their parents were included if they were fluent in Dutch and if the children were physically and mentally able to perform all tasks (see Euser et al., 2016 for more information on the recruitment procedure and full inclusion and exclusion criteria). Most children were living in families with a high (56%) or middle (37%) socioeconomic status (SES, based on the education level of the parents). Zygosity of the twins was determined by analyses of DNA samples collected by buccal swabs. When the DNA samples were missing (11%) zygosity was based on the zygosity questionnaire (Rietveld et al., 2000), which was filled out by the primary parent (the parent who spends the most time with the children). To create two independent groups we randomly assigned co-twins to either the test (sample A) or replication sample (sample B).

The final sample included 214 twin pairs, 59% monozygotic (MZ) and 41% dizygotic (DZ). Both test and replication samples consisted of 214 children (52% girls, *M* = 4.77 years, *SD* = 0.58, age range 3.86 – 6.54 years at the second wave of data collection). However, not all participants had valid data for all variables, therefore sample sizes vary somewhat for different analyses. For the POG, data were missing for seven children because they did not complete the task (test: n = 2; replication: n = 5). EEG data were missing for more children (test: n = 73, replication: n = 67), because of insufficient artifact-free EEG data (n = 50), technical problems (n = 29) or refusal to wear the EEG net (n = 61). Questionnaire data were missing for 12 children because the parents did not complete the questionnaires (test n = 6, replication n = 6), and donating data was missing when children did not complete the task (test n = 9, replication n = 10).

The local ethics committee and the Central Committee on Research involving Human Subjects in the Netherlands (CCMO; NL49069.000.14, Samen Uniek) approved of the study protocol. Informed consent was obtained for all participants prior to their involvement in the longitudinal study, for each twin both parents provided written informed consent. Families received a financial reimbursement after each visit and a small gift for the children.

Procedure

Participants took part in a longitudinal study with yearly visits. The current study includes data from the second wave of the data collection (n = 428 children). One week before the lab visit the parents received an e-mail asking them to complete online questionnaires, including the Strengths and Difficulties Questionnaire (SDQ; Goodman, Lamping, & Ploubidis, 2010) and the My Child Questionnaire (MCQ; Kochanska, DeVet, Goldman, Murray, & Putnam, 1994). The primary parent and the twins were invited for a lab visit with a total duration of approximately three hours. Each child was supervised by a research assistant who guided the child through the test session. Co-twins were randomly assigned to first complete either the block of behavioral tasks (including individual tasks and parent-child interaction tasks, results are presented elsewhere) or the block including EEG measures (including individual tasks, a resting baseline EEG measure and an EEG task measure). For the current study we used data from the resting baseline EEG measure only. Before starting the EEG measures the procedure was explained to parent and child. Next, the child was fitted with the electrode net. The EEG assessment consisted of a 3-minute resting baseline EEG measurement, followed by a task of approximately 15 minutes (see Van Wijk et al., 2017). After removal of the EEG-net, two behavioral tasks were performed, the Prosocial Owl Game and the Donating task.

Measures and Data Processing

Prosocial Owl Game. To measure prosocial behavior in response to social exclusion we used an adapted version of the four-player Prosocial Cyberball Game (PCG, Riem et al., 2013; Vrijhof et al., 2016; Van der Meulen et al., 2016, 2017). In our PCG version for early childhood the three virtual players used in the PCG are replaced with colorful cartoon owls, after which the game is called the “Prosocial Owl Game” (R. Damsteegt, Consortium on Individual Development, personal communication, March, 2015). The task consists of five stories in which owls are playing together in the playground: frisbee tossing, slide, ballgame, swing and spring rider (see Figure 1). The task has been programmed on a tablet and presents pictures with simple animations and audio instructions. The use of several playground stories helps the participants to remain motivated. The task starts with a fair game (frisbee tossing), in which none of the owls are excluded; all owls get equal turns. The next games are four exclusion games in which one owl is excluded and gets no turns from the other two owls. Each story consists of three trials and each trial shows three turns of the owls playing the game, in the fourth turn the participant can choose an owl to play next.

At the start of each story the three owls are introduced. Each story contains three different owls and they all have gender-neutral names consisting of four letters. In each story the excluded owl is shown at a different location (either in the middle, left or right of the screen) and with a different color to minimize the effect of the owl’s location or color on any compensating behavior. The order of the exclusion stories was randomized between participants. Children were presented with four exclusion games, including three trials each, leading to a possibility to compensate in twelve trials. Compensating behavior was coded as 1 for each trial in which the participant chose the excluded owl to play. The duration of the task was approximately 10 minutes.

At the end of the task we asked two exit questions that could be answered with ‘yes’ or ‘no’. The first question “Did you think the games were fair?” was answered by the child. The second question “Did the child notice the exclusion during the game?” was answered by the experimenter based on comments about the exclusion that the child had made during the game. Examples of comments that the children made during the game are “that owl did not receive the ball”, “why is that owl not allowed to go on the swing?”, or “that is not fair!”. The experimenter coded these as evidence that the child noticed the exclusion of one of the owls during the game.

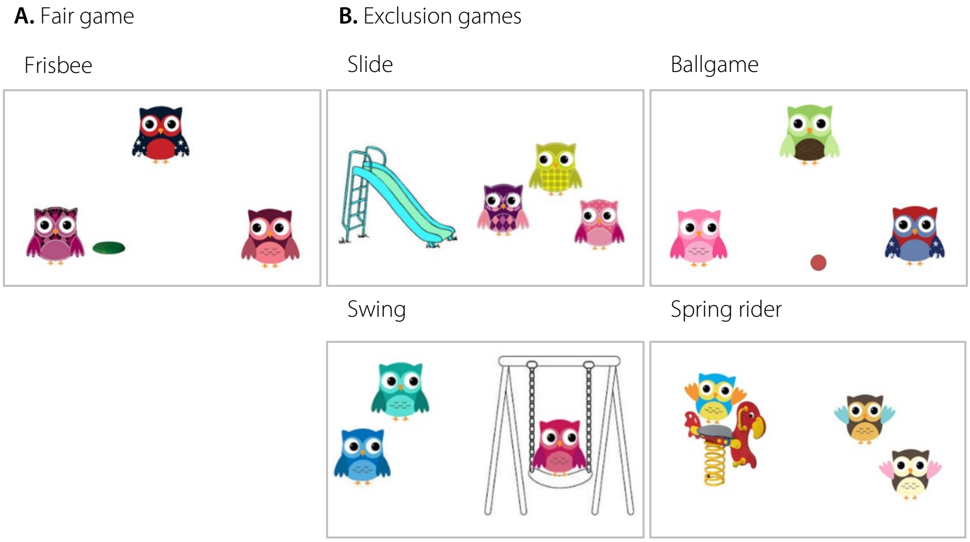

Figure 1. Prosocial Owl Game. (A) The first game is a fair game (frisbee tossing), in which none of the owls is excluded and they all get equal turns. (B) The next four games are presented in random order and consist of three exclusion trials were one owl is consistently (three turns) excluded and gets no turns. In all games the participants can pick an owl each fourth turn.

Donating task. Donating behavior was measured with an adapted version of the sharing task based on Knafo, Israel, and Ebstein (2011). After the POG, the participants received ten attractive stickers as a gift for doing well during the previous tasks, and an envelope. The experimenter explained to the child: "These ten stickers are for you. Tomorrow another child will visit the lab and perform the same tasks as you did today. However, that child does not get any stickers. You can decide to give stickers to the child who will visit us tomorrow. If you want to give stickers to the child who gets no stickers, you can put stickers in the envelope, and I will give the envelope to the child tomorrow. If you want to keep all the stickers for yourself then you can give me back an empty envelope. You may decide whether you give any stickers and if so, how many. I will check whether your brother/sister is done with the games in the other room and I will be back in a minute." After providing the instruction the experimenter left the room and kept an eye on the child via a live video that showed the room in which the child was. After one minute the experimenter went back to the child and asked "Are you ready with dividing the stickers? Can I have the envelope?". The number of stickers in the envelope was counted after the lab visit, in absence of the child.

The distributions of the numbers of donated stickers in both the test and replication samples were severely skewed to the right. To obtain a more evenly distributed variable we distinguished three categories (comparable to Wildeboer et al., 2017): children who did not donate any stickers (sample A: 50%; sample B: 43%), children who donated less than half of all stickers (1-4 stickers; sample A: 24%; sample B: 26%), and children who donated at least half of all stickers (5-10 stickers; sample A: 26%; sample B: 31%).

Questionnaires. We used the following parent reports on prosocial behavior and empathy of the child. Both parents (primary and other parent) completed five items of the Prosocial scale of the Strengths and Difficulties Questionnaire (SDQ, Goodman et al., 2010) and 13 items of the subscale Empathic concern, the prosocial response to another's distress, of the MyChild Questionnaire (MCQ, Kochanska et al., 1994). SDQ items were rated on a three-point scale ranging from *not true* (1) to *certainly true* (3). The MCQ had a five-point scale ranging from *untrue* (1) to *true* (5). The MCQ included an extra "not applicable" option when the behavior described in the item was not previously observed in the child, these items were coded as missing values and were not included in subscale scores. Some items were recoded in order to get higher scores reflecting higher levels of prosocial behavior or empathy.

We conducted a principal component analysis (PCA) on all 18 items completed by the primary parent and by the other parent in the test sample. Based on the scree plot and explained variance we identified two clear factors that together explained 36% of the variance. Two items from the MCQ ("May occasionally tease a pet if unsupervised" and "Feels good when good things happen to movie characters") scored low on both factors (loadings of < .3), maybe because they are less age-adequate, and were removed from further analyses. Based on the content of the items we named the first factor “Empathy” (including for example SDQ item “Is helpful if someone is hurt”) and the second factor “Contagion” (including for example MCQ item “Is upset by stories in which characters are hurt or die”). We followed the same procedure in the replication sample. The PCA showed that in this sample the same composition of items resulted in adequate factor loadings for the two factors *Empathy* and *Contagion*. The first factor ‘Empathy’ from the PCA included 11 items (see supplementary material, Table 1) and showed a good internal consistency in both samples (test: primary parent α = .84, other parent α = .85 and replication: primary parent α = .81, other parent α = .81). The second factor ‘Contagion’ included five items but one item (“My child seldom cries when seeing something sad on tv”) had to be removed because it did not fit compared to the other items (internal consistency with five items was lower than .60). The second factor with four items (see supplementary material, Table 1) showed marginal internal consistency (test: primary parent α = .70, other parent α = .66 and replication: primary parent α = .61, other parent α = .63). For each factor a mean score across the items was computed for both primary and other parent and for the test and replication sample separately. Before computing mean scores, SDQ items were first transformed to the same scale (1 to 5) as the MCQ items. The data showed five outliers (|z| < 3.29; three in the test sample and two in the replication sample) that were winsorized (Tabachnick & Fidell, 2006). The correlations between the primary and other parent were all significant (test sample: Empathy *r* = .48; Contagion *r* = .31; replication sample: Empathy *r* = .52; Contagion *r* = .25, all *p* < .01). Therefore we computed mean scores based on both parent’s ratings. When one of the parents did not fill out the questionnaires (n = 17 for the primary parent and n = 45 for the other parent) the score of the parent who did complete the SDQ and MCQ was used in further analyses. Both factors (Empathy and Contagion) were close to normally distributed and two outliers (|z| < 3.29, only in the replication sample) were winsorized (Tabachnick & Fidell, 2006).

Frontal EEG asymmetry. For the current study, we used the same procedure for data processing and analysis of the EEG data as previously described in Van Wijk et al., 2017. EEG was recorded during a 3-minute resting baseline. The child was instructed to alternatingly open and close his or her eyes for 30 seconds each (3x30 seconds eyes open and 3x30 seconds eyes closed). The computer played an audio message telling the child to close his/her eyes and displayed a drawing of closed eyes when the child had to close his/her eyes. After 30 seconds an audio message was played saying the child could open his/her eyes again. During the *eyes open* trials the child saw a color-changing dot on the screen to focus attention and avoid excessive eye-movements.

A 64-channel hydrocel geodesic sensor net and NetStation software (Electrical Geodesics, Inc.) with a NetAmps300 amplifier were used to record the EEG. To ensure a good signal each electrode was adjusted to keep impedances below 100 kΩ. To avoid fatigue, irritability and loss of attention in young children we minimized preparation time by adjusting and collecting data from only a subset of the electrodes (number in brackets): F3 [12], F4 [60], F7 [18], F8 [8], C3 [20], C4 [50], T7 [24], T8 [52], P3 [28], P4 [42], P7 [30], P8 [44], left [29] and right [47] mastoids, and two electrodes [62, 63] placed directly below the eyes. During recording the reference was Cz and data were low-pass filtered at the Nyquist frequency (i.e. 100Hz) for the sampling rate of 250 Hz.

After applying a 0.3 Hz high-pass filter (99.9% pass-band gain, 0.1% stop-band gain, 1.5 Hz roll-off) data were exported for further processing using Brain Vision Analyzer (BVA) 2.0 software (Brain Products, Inc). The EEG was low-pass filtered at 30 Hz (-3 dB, 48 dB/octave). The six 30-second trials were segmented into 2-second segments with 1-second overlap. Segments containing artifacts (i.e., segments in which the difference between the largest and smallest value was larger than 200 µV or in which the difference between the largest and smallest value within any 100 ms interval was smaller than 0.5 µV in any channel) were removed and bad channels were deleted from an individual dataset if the channel contained artifacts in more than 50% of segments. A fast Fourier Transformation (0.5 Hz resolution, 100% Hamming window) was used to compute power values (μV^2). Power values were averaged per condition over the artifact-free segments. The minimum requirement for a child's data to be included in further analyses was 28 segments per condition (equal to 56 seconds). On average 63 segments per condition were included (eyes closed: M = 61 [29 - 87]; eyes open: M = 65 [29 - 87]).

Power values were averaged across the frequency range of 6-10 Hz (alpha power in young children; Marshall, Bar-Haim, & Fox, 2002) to obtain alpha power for each condition. With a natural log transformation the data distributions were normalized. Frontal alpha asymmetry was computed by subtracting alpha activity over left frontal areas (electrode F3) from alpha activity over right frontal areas (electrode F4). The data showed seven outliers ($|z| > 3.29$) that were winsorized (Tabachnick & Fidell, 2006). There were 42 children with sufficient artifact-free EEG data for one condition only (eyes open n = 33 or eyes closed n = 9). To maximize the number of children in the analyses and because of the high correlation between the *eyes open* and *eyes closed* conditions ($r = .88$, $p < .01$), we estimated the value of the missing condition based on the value of the other condition using the regression equation obtained in the subsample of children with sufficient data for both conditions in the total sample (both test and replication sample, n = 246). Using this method, data of 42 children could be imputed and included in the analyses. Furthermore, because of the high correlation between the conditions *eyes open* and *eyes closed* we decided to average across the two conditions to obtain one value of FA per child, which we used in all subsequent analyses.

Data analyses

Preliminary analysis. Compensating behavior in the Prosocial Owl Game was analyzed using SPSS 23. First we checked whether the participants showed any systematic pattern of choice during the fair game by examining the percentages of expected and observed choices of each owl with chi-square tests. Next, we examined the pattern of compensating behavior during the exclusion games. Compensating scores per trial were summed over the four exclusion stories, leading to four variables: first, second, and third trial, and second and third trials combined. Monozygotic (MZ) versus dizygotic (DZ) within-twin correlations were computed to see whether compensating behavior may be influenced by genetic in addition to environmental factors, as higher MZ correlations than DZ correlations suggest genetic influences. To estimate heritability we computed Falconer's equations (Falconer & Mackay, 1996), with heritability defined as $h^2 = 2 \times (r_{MZ} - r_{DZ})$, in case of (non-significant) negative correlations we set the correlation to zero. In addition, we examined whether age, gender or SES were related to compensating behavior during the POG using respectively Pearson's correlations, independent samples t-tests and one-way ANOVAs, because of the potential confounding effect of these background variables.

Repeated measures and correlations. Differences in compensating behavior over the trials were investigated with repeated measures analysis of covariance (rmANCOVA). The results of the POG were correlated with FA to examine associations with approach-withdrawal tendencies as reflected by hemispheric differences in brain activity. Last, to examine whether compensating behavior was related to other prosocial behavior measures, POG compensating behavior was correlated with donating behavior and parent-reported Empathy and Contagion.

Results

Preliminary Analyses

Fair versus exclusion games. In the fair game we expected an equal chance of 33% for each owl to be chosen. Table 1 shows an overview of the observed percentages in the fair game in the test and replication samples. Chi-square tests revealed that there was no preference for a specific owl during the fair games in the test sample in the first or third trials ($p > .05$), but there was a small preference for the left owl in the second trial ($\chi^2(2) = 6.24$, $p = .04$). In the replication sample there seemed to be a small preference for the middle owl in the first trial ($\chi^2(2) = 6.47$, $p = .04$), whereas the second and third trials did not show a preference for a specific owl ($p > .05$). Overall, we concluded that the data showed no systematic pattern of choice during the fair game. In the exclusion games, participants showed a clear preference for the excluded owl, see Table 2. We summed the choices of the children for each owl (the excluded owl and the other two owls) over the games and chi-square tests confirmed that participants chose the excluded owl more often than expected by chance (test: χ^2 *(2)* [158,54 – 694,43], p < .01; replication: χ^2 *(2)* [140,97 – 743,91], p < .01). This preference for the excluded owl indicated that the children showed prosocial compensating behavior. Especially during the first trial, the vast majority of the children (73 – 81%) showed compensating behavior, indicating low variance in compensating behavior between individuals on the first trials.

Twin correlations. Because the first trials of the POG did not show much variance between the children, we did not compute within-twin correlations for the first trials. In the second and third trials MZ twin correlations were more than twice as large as DZ twin correlations (second trial: r_{MZ}= .30, p < .01; r_{DZ} = -.06, p = .59, h^2 = .60); third trial: r_{MZ}= .38, p < .01; r_{DZ} = -.01, p = .92, h^2 = .76), which suggests a substantial genetic influence on compensating behavior. We also computed a variable that combined the second and third trials (with a compensation score ranging from 0-8). As expected, MZ twin correlations were more than twice as large as DZ twin correlations and the heritability estimate was large (r_{MZ}= .49, p < .01; r_{DZ} = -.07, p = .56, h^2 = .98).

Table 1. Pattern of chosen owls during Fair Game in percentages.

Sample	Trial	Owl A	Owl B	Owl C	χ^2
Test	1	26	39	38	5.02
	2	27	32	41	6.24*
	3	39	32	29	3.58
Replication	1	26	44	33	6.47*
	2	28	35	36	2.48
	3	37	29	34	2.19

Note: chi-square test shows differences between observed versus expected (i.e. 33%, equal distribution between owls) values. ** p < .01; *p < .05

Table 2. Pattern of compensation behavior (percentages of choosing the excluded owl).

Sample	Trial	Game 1	Game 2	Game 3	Game 4
Test	1	76	81	73	73
	2	52	55	53	53
	3	50	60	55	52
Replication	1	81	74	79	77
	2	47	57	57	50
	3	59	51	57	57

Exit questions. At the end of the POG we asked the children whether they thought the games were fair. The majority of the children responded with 'yes' (test: 57%; replication: 53%), about one-third of the children responded with 'no' (test: 30%, replication: 33%). Data were missing for the rest of the children. Independent samples t-tests showed that there were no significant differences in compensating behavior between children who did or did not think the games were fair (test: $t(203)$ = [-0.89 - 0.97, $ps > .05$, replication $t(202)$ =[0.24 - 1.51, $ps > .05$). Approximately half of the children spontaneously mentioned the exclusion during the game (test: 47%, replication: 47%). The other children did not comment on the exclusion. When the child mentioned the exclusion during the task, the child compensated significantly more in the second trial (test: $M = 2.4$, $SD = 1.6$; replication: $M = 2.3$, $SD = 1.3$) compared to children who did not comment on the exclusion (test: $M = 1.9$, $SD = 1.3$; replication: $M = 1.9$, $SD = 1.2$; test: $t\,(210) = -3.02$, $p < .01$, replication: $t\,(207) = -2.66$, $p < .01$). This effect was not found in the first or third trials ($ps > .05$).

Gender, age and SES. No gender difference was found in the second or third trial (test and replication: all $p > .05$, d = [0.01 – 0.17]). However, boys and girls were significantly different in their compensating behavior during the first trial ($t(210) = -2.46$, $p <.05$, $d = 0.34$); girls compensated more ($M = 3.19$, $SD = 0.93$) than boys ($M = 2.87$, $SD = 0.96$) in the test sample, but not in the replication sample. Older children showed more compensating behavior than younger children in the second trial, but only in the test sample ($r = .23$, $p < .01$, all other $r \leq .13$, $ps > .05$). In both samples, parental SES was not related to compensating behavior (F (2, 210) = [0.17 – 2.29], $ps > .05$, η_p^2 = [.00 – .02]). Based on these results, we included gender and age as covariates in the rmANOVA and in the correlations with other prosocial measures.

Repeated Measures and Correlations. Results of the rmANCOVA showed a main effect of trial in both the test and replication sample (test: $F(2,211) = 54.42$, $p < .01$, $\eta_p^2 = .21$; replication: $F(2,208) = 60.22$, $p < .01$, $\eta_p^2 = .23$). Planned post hoc pairwise comparisons showed that children compensated significantly more in the first trial (test: $M = 3.04$, $SD = 0.95$; replication: $M = 3.11$, $SD = 0.99$) than in the second (test: $M = 2.15$, $SD = 1.25$; replication: $M = 2.11$, $SD = 1.29$, $p < .01$) and third trial (test: $M = 2.16$, $SD = 1.17$; replication: $M = 2.24$, $SD = 1.08$, $p < .01$), see Figure 2. No significant difference was found between the second and third trial ($p = 1.00$). On the contrary, these trials were significantly correlated (test: $r = .25$, $p < .01$, replication $r = .34$, $p < .01$), which supports their combination into one POG score.

The outcomes of the POG were not related to frontal asymmetry (all $r < .15$, $p > .05$) or to other measures of prosocial behavior (donating and parent-reported Empathy and Contagion, $rs < .15$, $ps > .05$), see Table 3 for an overview of the correlations.

Table 3. Partial correlations (including covariates gender and age), mean scores and standard deviations for all variables.

	1	2	3	4	5	6	7	8	M	SD
1 POG trial 1		.03	.22**	.15*	-.04	.11	.00	.11	3.11	0.99
2 POG trial 2	.10		.34**	.85**	.06	.01	-.02	.10	2.11	1.29
3 POG trial 3	.26**	.25**		.78**	.01	.00	-.01	-.03	2.24	1.08
4 POG trial 2+3	.22**	.80**	.78**		.04	.00	-.02	.05	4.35	1.94
5 Donating behavior	-.01	-.01	.09	.05		-.04	.01	.07	1.89	0.86
6 Reported empathy	-.02	-.11	-.10	-.13	-.01		.20**	-.04	4.18	0.56
7 Reported contagion	.05	.00	-.04	-.02	-.04	.29**		-.03	2.87	0.79
8 Frontal asymmetry	-.06	.02	.13	.09	-.17*	-.03	-.08		-0.11	0.23
M	3.04	2.15	2.16	4.31	1.76	4.24	2.92	-0.09		
SD	0.95	1.25	1.17	1.92	0.84	0.53	0.80	0.24		

Note. Correlations for the test sample are presented below the diagonal, and correlations for the replication sample are presented above the diagonal. Means and standard deviations for the test sample are presented in the horizontal rows and for the replication sample in vertical rows. Sample size for variables 1 - 7 ranged from 204 – 212, sample size for frontal asymmetry were: test: n = 141; replication: n = 147. ** *p* < .01; **p* < .05

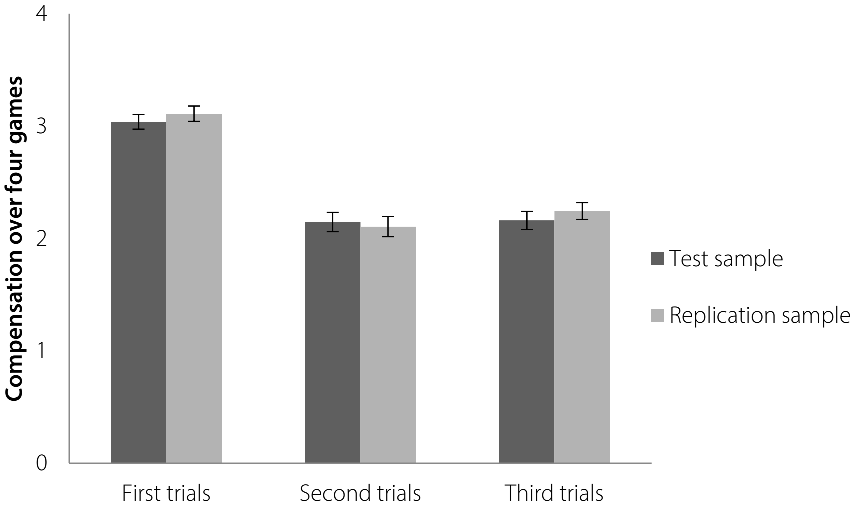

Figure 2. Compensating behavior (amount of choosing the excluded owl). In both test and replication sample children compensate more in the first trial compared to the second and third trial per game (*p* < .001; error bars represent standard errors).

Discussion

The current study examined compensating behavior in reaction to social exclusion in 4 – 6-year-olds by using a newly developed task: the Prosocial Owl Game (POG). In line with previous studies using the Prosocial Cyberball Game (PCG; Riem et al., 2013; Vrijhof et al., 2016; van der Meulen et al., 2016, 2017), results showed that in general children respond prosocially after social exclusion by choosing the excluded owl more often than expected by chance. During the first trials children compensated significantly more often than during the second and third trials – in fact there was not much inter-individual variance in responses at the first trials, indicating that the exclusion of one of the owls had been (consciously or unconsciously) registered. There was more individual variability in compensating behavior in the second and third trials, implying that individual differences only appear later in the game. FA was not related to compensating behavior during the POG and neither were parent-reported prosocial behavior or observed donating behavior. Results were similar in the test and replication sample.

The goal of the POG was to measure prosocial compensating behavior in response to social exclusion. On the first trial of each game we found little variance between the children, the majority of children showed compensating behavior by choosing the excluded owl. When the game progressed, there was more variation between the children in their compensating behavior as a smaller proportion of children compensated for the social exclusion in the second and third trials. Variation in the second trial was related to whether or not the children mentioned the social exclusion during the POG. Children who spontaneously said something about the exclusion during the game showed more compensating behavior in the second trial than children who did not mention the exclusion. These children might have been surprised that the social exclusion by the other players continued and responded both verbally and behaviorally by including the excluded owl in the game. However, individual differences in the third trial could not be explained by whether or not the children mentioned the social exclusion. Overall, our findings suggest that more than one trial is necessary to elicit individual differences in prosocial behavior. This is in line with a recent meta-analysis on the relation between the observability of a prosocial act and the degree of displayed prosocial behavior, which showed that there was a stronger positive effect on observable prosocial behavior when the measurement was repeated compared to a single measurement (Bradley, Lawrence, & Ferguson, 2018). Related to this, both the second and the third trials of the POG showed stronger MZ correlations than DZ correlations for compensating behavior, suggesting heritability of prosocial compensating behavior, which is in line with the results of other studies on prosocial behavior (Knafo-Noam, Vertsberger, & Israel, 2018).

Individual differences in compensating behavior during the POG could not be explained by gender or age. Age was only related to compensating behavior in the second trial of the test sample (older children compensated more), but this effect was not found in the replication sample. However, the age range was quite small, and age-related effects might occur over a broader age range. Although gender and age effects were not replicated, we did correct for gender and age in further analyses as other studies investigating prosocial behavior found inconsistent results as well. A review by Rose and Rudolph (2006) showed that gender differences, in favor of girls, are mostly found when subjective measures of prosocial behavior are used (either self-, peer- or teacher reports). In addition, observational studies indicated that these gender differences seem to become more consistent with age (Rose & Rudolph, 2006). Some argue that stereotypic gender roles affect the findings on gender differences in subjective measures of prosocial behavior, as girls are generally expected to be more prosocial than boys (Eisenberg et al., 2015). With regard to compensating behavior, previous research with adolescents did not find gender or age effects on the PCG (Vrijhof et al., 2016), suggesting that compensating behavior in reaction to social exclusion is less influenced by expectations and might be a more valid measure of prosocial behavior across ages and gender.

Compensating behavior in reaction to social exclusion was not related to FA. Also, FA was unrelated to parent-reported Empathy and Contagion and observed donating behavior. Some other studies involving infants (Paulus et al., 2013) and adults (Huffmeijer et al., 2012) reported an association of relatively greater left frontal brain activity with prosocial behavior. The alpha frequency band, underlying FA, is subject to developmental changes (Saby &

Marshall, 2012), and this may account for different results in studies on different age groups. In children the estimates of the appropriate alpha frequency bandwidth (progressing from 6 – 9 Hz in infancy to 8 – 12 or 13 Hz in late adolescence and adulthood) are based on developmental changes in peak frequencies (Marshall et al., 2002). However, empirical studies proving that the 6 – 10 Hz frequency band indeed represents deactivation of cortical tissue and is thus inversely related to relatively greater brain activity in young children are lacking. As mentioned previously (Van Wijk et al., 2017), studies examining the development of the EEG frequency composition, 'alpha' bandwidth, and FA in children are thus badly needed.

Compensating behavior during the POG was not related to the other, more conventional measures of prosocial behavior. Although such associations would point to convergent validity of the measure, the absence of such associations does not indicate a lack of validity. Empirical studies have repeatedly shown that prosocial behavior is a multidimensional construct, and that outcomes are dependent on the context and on the type of prosocial behavior measured (Paulus, 2018; Padilla-Walker & Carlo, 2015). As a consequence, other studies failed to find associations between different prosocial responses in infants as well (Dunfield, Kuhlmeier, O'Connell, & Kelley, 2011; for a review see Thompson & Newton, 2013). In addition, compensating behavior during the PCG was not related to self-reported prosocial behavior in adolescents (Vrijhof et al., 2016) or self-reported empathy in adults either (Van der Meulen et al., 2016). The fact that we obtained substantial heritability estimates for prosocial POG behavior indicates that results do not merely reflect measurement error. Further research is necessary to explain exactly what factors underlie individual differences in prosocial compensating behavior.

Our study has some limitations that could also be addressed in future studies. First, the external validity of the POG should be further investigated. In general laboratory tasks are under debate because it is difficult to ensure that findings obtained using experimental tasks in laboratory settings are generalizable to real life situations (e.g., Winking & Mizer, 2013). The POG is based on the PCG which in turn is a variant of the classic Cyberball game. Cyberball is based on a real life experience (Williams & Jarvis, 2006) and even when participants know that they are being excluded by a computer instead of real-life players they still feel ostracized (Zadro, Williams, & Richardson, 2004). In addition, online social exclusion shows similar results as in-person social exclusion (Filipkowski & Smyth, 2012), which may be considered as support for the external validity of Cyberball. During the POG the children noticed the social exclusion, similar to Cyberball. Like the PCG, the POG included the possibility for the child to compensate for the exclusion. More research is necessary to ensure that compensating behavior during computerized games is similar to real-life prosocial compensating behavior. Second, about 44% of the children provided no usable FA data (test: n = 73, replication: n = 67), an attrition rate that is common in EEG research with young children (Bell & Cuevas, 2012). Future studies should search for ways to improve the quality and quantity of EEG data in early childhood.

We also point out some significant strengths of the study. First, our newly developed task has several advantages compared to other prosocial measures. Other observational tasks often use actors in order to provoke helping, sharing or caring behavior. Minor differences in acting or physical appearance of the actor might influence the behavior of the child. Therefore we standardized the procedure of the POG by programming the game on a tablet with animations and audio instructions to create a more objective task that requires minimal involvement of the experimenter. In addition, we randomized the position and color of the excluded owl which ensured that symmetry (e.g. Vrijhof et al., 2016) or color preferences of the participant did not influence compensating behavior. Hence, we suggest that the POG is an objective and feasible task to measure non-costly prosocial compensating behavior in reaction to social exclusion. Second, we used a replication design. The importance of replicability has been a hot topic lately because of the need to find a way to overcome bias and error in science (Pashler & Wagenmakers, 2012). In our study we used matched twin samples and showed that most outcomes were replicated, indicating that the outcomes of the POG are consistent and reliable. The test and replication sample were created by randomizing each co-twin to one of the two samples. This procedure optimizes replication because the two samples are similar in age, gender and family background. Another advantage of the twin sample was that we could compute within-twin correlations to indicate genetic influences on prosocial compensating behavior.

In conclusion, the current study showed that 4 – 6-year old children compensated for social exclusion in the "Prosocial Owl Game" task. The vast majority of children showed compensating behavior in the first trial of each game and individual differences emerged in the second and third trial of each game. Individual differences in prosocial compensating behavior could not be explained by FA, parent-reported prosocial behavior or observed donating behavior of the child. Future research should examine factors that influence prosocial compensating behavior in reaction to social exclusion. The high MZ correlations compared to DZ correlations of the POG suggest that genetic factors play a role. This study shows that the POG can be used to measure prosocial compensating behavior in young children in a similar way as the PCG is used with older children, adolescents and adults (Riem et al., 2013; Vrijhof et al., 2016; van der Meulen et al., 2016, 2017). The POG therefore facilitates developmental studies of prosocial compensating behavior across ages and with longitudinal designs.

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CHAPTER 4

Social judgments, frontal asymmetry, and aggressive behavior in young children: A replication study using EEG

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Abstract

Early in their lives young children are confronted with social judgments by peers. Previous studies have shown that in adults negative social judgments are associated with more aggressive behavior. However, little is known about the relation between social judgments and aggressive behavior, or the underlying neurocognitive mechanisms, in early childhood. We developed the Social Network Aggression Task - Early Childhood (SNAT-EC) to examine the mediating role of frontal EEG asymmetry in the relation between social judgment and aggressive behavior in 4–6 year old children. To replicate our findings, we included three samples: a pilot sample, test sample 1 and test sample 2 (total N =78). In the SNAT-EC, children receive positive, negative and neutral social judgments about their chosen cuddly animal by same-aged unfamiliar peers. EEG was acquired to measure frontal asymmetry during the processing of social judgments. Aggressive behavior was measured as the duration of a button press with which children could destroy balloons of the judging peer, thus reducing the number of remaining balloons for that peer. We used a within-subject mediation model to test whether frontal asymmetry mediated the effect of social judgment (negative vs. positive) on aggressive behavior. Results show that the SNAT-EC robustly elicits more aggressive behavior in response to negative social judgments about the cuddly animal compared to positive judgments. Meta-analysis revealed a large combined effect size (r = .42) for the relation between negative (vs. positive) social judgments and aggressive behavior. However, frontal asymmetry in response to the social judgments did not mediate the relation between social judgment and aggressive behavior. Future studies should search for other neural mediators to bridge the brain-behavior gap between social judgments and aggressive behavior, in particular in early childhood.

Keywords: Social judgments, aggression, frontal EEG asymmetry, early childhood, replication

Introduction

From early childhood onwards, children are confronted with social judgments from peers that imply social acceptance or rejection (Coie et al., 1982). According to the social belongingness hypothesis (Baumeister and Leary, 1995), social acceptance is important for humans, and experiencing negative social judgments at a young age has a great impact on mental health and stress levels later in life (Lereya et al., 2015; Newman et al., 2010). In addition, a longitudinal study using sociometric interviews and teacher reports showed that peer rejection is associated with an increase in aggressive behavior in schoolage children (Dodge et al., 2003). A study by Buckley and colleagues (2004) further highlights the role of negative emotions. These authors showed that receiving negative social judgments evokes negative emotional feelings, such as anger and sadness, that in turn can lead to aggressive behavior (Buckley et al., 2004). However, the direct effects of social judgments on aggression in early childhood have not yet been examined with experimental paradigms. It is important to investigate such direct effects to determine whether negative social judgments immediately cause aggression. Also, using appropriate measures, experiments can provide important insights into the underlying neurocognitive mechanisms that mediate a relation between social judgment and aggressive behavior. The current study investigated the neural and behavioral responses to positive, negative and neutral social judgments in 4- to 6-year-old children with the newly developed Social Network Aggression Task for Early Childhood (SNAT-EC).

The neural mechanisms involved in the processing of social judgments can be investigated using the social judgment paradigm from Somerville and colleagues (Somerville et al., 2006). In this task, participants are expectedly or unexpectedly accepted or rejected by peers. Imaging studies of social judgment processing in adult participants provided some insights into the brain structures involved in processing social rejection (a.o., vACC, striatum, several regions of prefrontal cortex regions (Gunther Moor et al., 2010; Somerville et al., 2006)). The processing of social judgments is further investigated in adults (Achterberg et al., 2016) and 7–10 year old children (Achterberg et al., 2017) by adding a behavioral response: participants could blast a loud noise to the judging peer after receiving a social judgment. Participants reacted more aggressively by blasting louder noises after receiving a negative social judgment than after a neutral or positive social judgment (Achterberg et al., 2016). However, the authors did not test whether effects of social judgments on brain activity mediated effects on aggressive behavior. Thus it remained unclear whether neural activity in response to negative judgments explains aggressive behavior, especially in early childhood.

Here we study asymmetric frontal cortical activity as a potential neural mechanism of aggressive behavior in response to social judgments in early childhood. Asymmetric frontal cortical activity reflects the difference in activity of the left and right frontal hemispheres and can be measured using electroencephalography (EEG). Because higher power in the EEG alpha band reflects deactivation of cortical tissue (Cook et al., 1998; Laufs et al., 2003), higher alpha power over the left than over the right frontal cortex reflects relatively greater activity of the right frontal areas. Conversely, higher alpha power over the right than the left frontal cortex reflects relatively greater activity of the left frontal cortex. The motivational direction model explains frontal asymmetries in terms of approach-withdrawal motivation (Harmon-Jones et al., 2010). Relatively greater left frontal brain activity reflects a tendency toward approach behavior and relatively greater right frontal brain activity reflects a tendency toward withdrawal behavior. For example, feelings of aggression, an approach–related emotion, have been associated with greater left than right frontal brain activity (Harmon-Jones, 2004, 2007; Harmon-Jones and Allen, 1998; see also Harmon-Jones et al., 2010).

Both trait levels as well as state-related variations in approach-withdrawal motivation contribute to measures of frontal asymmetry (Coan and Allen, 2004). Condition differences in frontal asymmetry, as well as changes relative to a baseline measure can be used to track state-related fluctuations (Hagemann et al., 2005; Harmon-Jones and Sigelman, 2001; Verona et al., 2009). For example, Harmon-Jones and Sigelman (2001) found that anger provoking insults from peers resulted in relatively greater left frontal activity. To measure aggressive behavior in response to these insults, participants could administer unpleasant beverages like vinegar or hot sauce mixed with water to the insulting peers. The authors found that participants who showed more aggressive behavior after an insult also showed greater relative left frontal activity (Harmon-Jones and Sigelman, 2001). Such studies suggest that greater relative left frontal activity may mediate the association between anger evoking stimuli and aggressive behavioral reactions. In fact, frontal asymmetry has been suggested as a likely mediator of behavioral responses more generally: the effect of a stimulus on behavior is suggested to come about through frontal asymmetry and associated approach or withdrawal motivation (Coan and Allen, 2004). However, to the best of our knowledge, the mediating role of frontal asymmetry in responses to different stimuli in a within-subject design has not been examined yet.

In sum, previous studies including older children and adults have suggested that there are relations between social judgment, aggressive behavior and neural activity: negative social judgments lead to more aggressive behavior and several brain regions seem involved in processing social judgments (a.o., vACC, striatum, several regions of prefrontal cortex regions (Achterberg et al., 2016, 2017; Gunther Moor et al., 2010; Somerville et al., 2006)). However, whether these relations are already present in early childhood remains unknown. Moreover, as far as we know, no study to date has directly assessed whether neural processes mediate effects of social judgments on aggressive behavior. The association of relative left frontal asymmetry with approach motivation and feelings of anger and aggression (e.g., Harmon-Jones et al., 2010; Harmon-Jones and Sigelman, 2001) make it a likely candidate. The current study therefore examines whether frontal asymmetry in response to social judgments mediates the relation between social judgments and aggressive behavior in 4–6 year-old children. For this purpose, we developed the Social Network Aggression Task – Early Childhood (SNAT-EC) in which children received positive, neutral, and negative social judgments from same-aged unfamiliar peers. To measure aggressive behavior, in response to these social judgments, children could destroy balloons of the judging peer by pressing a button. The duration of the button press, reflecting the number of balloons destroyed, was used as the measure of aggression.

To test the validity of our task and the replicability of the outcomes (Collaboration, 2015; Pashler and Wagenmakers, 2012), we used three different samples: a pilot sample, test sample 1 and test sample 2. The pilot sample was independent from the two test samples. The two test samples consisted of same-sex twin pairs. Each co-twin was randomly assigned to either test sample 1 or test sample 2. Finally, we combined the results from each sample in a meta-analysis.

Based on previous findings (Achterberg et al., 2016; Dodge et al., 2003) we expected that children would react more aggressively after a negative social judgment compared to a positive or neutral social judgment. Furthermore, we hypothesized that the effects of social judgment on aggressive behavior would be mediated by frontal asymmetry: we expected that greater left frontal brain activity in response to negative social judgments would explain increased aggression after these judgments. Last, we expected to replicate the results from the pilot sample in the two test samples.

Materials and methods

Participants

The pilot sample included 13 opposite-sex twin pairs and 24 singletons, aged 4–7 years. Singletons were recruited at two elementary schools in the Leiden area. Opposite-sex twins were recruited via municipal authorities in the western part of the Netherlands. The two test samples included 50 same-sex twin pairs, aged 4–5 years, and consisted of the first 50 families who participated in the larger, longitudinal study of the Leiden Consortium on Individual Development (L-CID; Euser et al., 2016)). The families with same-sex twins were recruited via municipal authorities in the western part of the Netherlands. Twins and their parents were included if they were fluent in Dutch. Exclusion criteria for all participants were known disabilities or neurological impairments (e.g. congenital disability, psychological disorder, chronic illness, hereditary disease, or a visual or hearing impairment). Each co-twin was randomly assigned to either test sample 1 or test sample 2.

Some participants were excluded from the analysis due to insufficient artifact-free EEG data, too many invalid behavioral trials, technical problems or not enough trials seen (pilot N = 17, test sample 1 N = 15, test sample 2 N = 13). In addition, some children refused to wear the EEG-net (pilot N = 10; test sample 1 N = 11, test sample 2 N = 9). Characteristics of the included and excluded participants are shown in Table 1. The final pilot sample consisted of 21 children (8 girls, M = 6.02 years, SD = .73, 17 single children and 4 twin children), the final test sample 1 consisted of 27 children (16 girls, M = 5.16 years, SD = .38) and the final test sample 2 consisted of 30 children (14 girls, M = 5.12 years, SD = .45). The difference between included and excluded children was only significant for age in the pilot sample (pilot sample: t (48) = 7.03, p<.01; test sample 1: t (48) = 1.72, p = .09; test sample 2: t (48) = 1.27, p = .21). No significant gender differences were found between included and excluded children (pilot sample: X2 (1, N = 50) = .51, p = .47; test sample 1: X2 (1, N = 50) = .65, p = .42; test sample 2: X2 (1, N = 50) = 1.62, p = .20).

Participating children received a small gift and the caregiver received a financial reimbursement. Written informed consent was obtained from both caregivers. Study procedures were approved by the local ethics committee and the Central Committee on Research Involving Human Subjects in the Netherlands.

Procedure

The lab visit consisted of electroencephalogram (EEG) measures during the Social Network Aggression Task – Early Childhood (SNAT-EC), a baseline EEG measurement, and several behavioral tasks (results presented elsewhere). Twins were invited to the lab together; each co-twin was randomly assigned to the EEG block or the behavioral block as their first task. At the start of the EEG block the experimenter explained the EEG procedure to the parent and the child. Next, the child was fitted with an electrode net. After a 3-min resting (non-emotional) baseline EEG measurement the SNAT-EC was explained to the child. Participants were instructed how to destroy the balloons with a button press. Then the experimenter

Table 1. Characteristics of the samples.

	Pilot	Test 1	Test 2
Final Sample			
N	21	27	30
% girls	38%	59%	47%
Mean age in years (SD)	6.02 (.73)[a]	5.16 (.38)	5.12 (.45)
Age range	4.51 - 7.04	4.36 - 5.65	4.28 - 5.68
Excluded from sample			
N	29	23	20
% girls	48%	48%	65%
Mean age in years (SD)	4.77 (.54)[a]	4.95 (.48)	4.97 (.41)
Age range	4.21 – 6.41	4.28 – 5.68	4.36 – 5.50
Excluded due to (N):			
Refusing EEG-net	10	11	9
Technical problems	3	7	6
Invalid behavioral trials	5	4	3
Eyes off-screen (>50%)	4	-	-
EEG artifacts	7	1	2

[a] An age difference between final and excluded sample was found (t(48)=7.03, $p < .01$)

explained each social judgment to the child. To make sure that the child understood the judgments, we asked the child to repeat the meaning of each judgment. After 6 practice trials the SNAT-EC began. The total duration of the SNAT-EC was approximately 20 min after which the EEG recording was stopped. To motivate the children during the EEG measurement children received three stamps on a card: one after putting on the EEG net, one during a break (after 30 trials), and one at the end of the task.

Social Network Aggression Task – Early Childhood

To measure behavioral and neural responses to social judgments, we used an adapted version of the social evaluation paradigm developed by Somerville and colleagues (Somerville et al., 2006), which we called the Social Network Aggression Task – Early Childhood (SNAT-EC). In our version, children were not judged on personal characteristics but on a cuddly animal they had chosen as their favorite (see below). From an ethical perspective, rejection of the cuddly animal was preferred to rejection of the child him-/herself. In the SNAT-EC children could destroy balloons of the peer who had judged their cuddly animal as a measure of aggressive behavior.

Three weeks prior to the lab visit the children were asked via an e-mail to the primary caregiver to choose one out of five (pilot group) or four (test groups)[11] cuddly animals (see Fig. 1A). The cuddly animal was sent to the children's home two weeks before the lab visit to give the children time to get attached to the cuddly animal. During the lab visit participants were told a cover story explaining that other peers had judged their cuddly animal. Peers' feedback on the cuddly animal could be positive ("I like your cuddly animal"), negative ("Your cuddly animal is stupid") or neutral ("I don't know whether I like your cuddly animal"). In addition, participants were told that each peer had ten balloons. After receiving each peer's feedback on the computer screen, the participants could destroy the peers' balloons by pressing a button. The longer they pressed the button, the more balloons would be destroyed. Before the task started we explained to the participants that they had to press the button on each trial and that they should press the button very briefly if they did not want to destroy any balloons. The button press was practiced in 6 training trials during which the participants received feedback from the experimenter if necessary.

Feedback stimuli combined a judgment with a picture of the peer that supposedly provided the judgment. The pictures of the judging peers were created by morphing photographs of children to create a picture of a non-existing child matching with the age of the participants. This way, there was no chance that the participant would recognize a judging peer. Photographs were taken from young children at primary schools in two cities in the Netherlands. These photographs were morphed (using Abrosoft FantaMorph, version 5) with photographs of children from a database of Leiden University and Nijmegen University

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[1] Based on our experience from the pilot study we decided to let the children choose one out of four cuddly animals instead of five, because some cuddly animals were more popular than others and this way we could change the collection when one cuddly animal was out of stock.

(Langner et al., 2010). Pictures (20 × 28 mm) were placed inside a figure of a green thumb up (42 × 51 mm, positive), a red thumb down (42 × 51 mm, negative) or a grey oval (42 × 47 mm, neutral), resulting in 20 positive, 20 negative, and 20 neutral feedback stimuli respectively (see Fig. 1B). Stimuli were matched for luminance. Gender of the judging peers was equally divided over the three feedback types and during the task the judgments were presented in pseudorandom order with the restriction that the positive and neutral judgments could not be presented more than four times in a row and a negative judgment was never followed by another negative judgment.

For the pilot group the SNAT-EC was divided into two parts: the first part consisted of 90 observation trials (in which the child could not respond to the judgments) and the second part consisted of 60 action trials in which the participants could destroy the peer's balloons after seeing the judgment. After the pilot study we decided to shorten the task by leaving out the 90 observation trials to improve data quality during the action trials. For all samples the 60 action trials were used for data-analysis.

Each trial started with a fixation cross with a jittered duration of 500–1500 ms followed by a social judgment (positive and negative: 4.00 × 4.86° visual angle; neutral: 4.00 × 4.48° visual angle) for 4000 ms in the pilot group and for 2000 ms in the test groups[2], see Fig. 1C. Then another fixation cross was presented (duration 500–1500 ms, varying randomly) and thereafter a picture showing ten balloons (7.13 × 7.59° visual angle) appeared on the screen. Participants could destroy the balloons by pressing a button that was placed in front of the participant. After each 400 ms one balloon popped with a maximum of 9 balloons (4000 ms). Participants were instructed to start pressing the button as soon as possible and to release the button when they destroyed the number of balloons they wanted to destroy. To make sure each trial had the same duration, the image showing the remaining balloons stayed on screen for the remainder of the 4000 ms period after participants released the button. After

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[2] We used the pilot group to test for potential effects of stimulus duration on frontal asymmetry. Because frontal asymmetry over 4000 ms did not differ from frontal asymmetry over 2000 ms, we decided to shorten stimulus presentation in the test groups.

every 10 trials the participants had a 10-second break. After 30 trials there was a longer break (approximately 1 min).

Behavioral data for each subject was obtained by computing the mean pressing time per condition. Trials on which the participant did not press the button or failed to press it within 2000 ms were excluded. Eight trials per condition was considered a minimum to compute the mean pressing time.

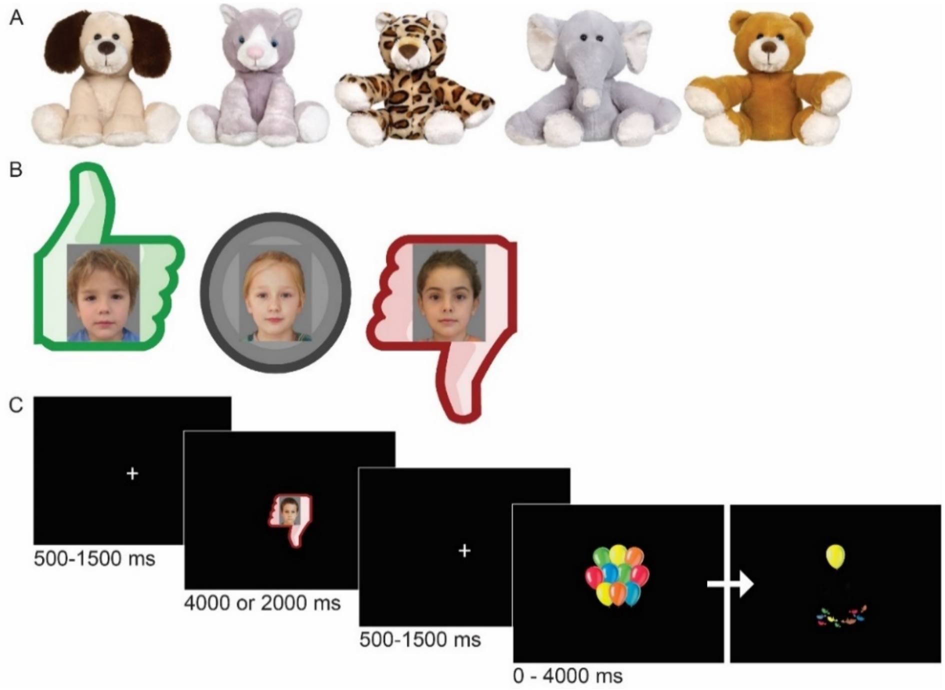

Figure 1. Social Network Aggression Task – Early Childhood (SNAT-EC). A) Selection of five cuddly animals from which each child chose one. B) The social judgments: positive, neutral and negative. C) Trial procedure of SNAT-EC.

EEG recordings

The EEG was recorded using a 64-channel hydrocel geodesic sensor net and NetStation software (Electrical Geodesics, Inc.). As it is important to minimize preparation time (each electrode needs to be adjusted to ensure a good connection) in order to avoid fatigue, irritability and a loss of attention in young children we decided to collect data from only a subset of the electrodes (number in brackets): F3 [12], F4 [60], F7 [18], F8 [8], C3 [20], C4 [50], T7 [24], T8 [52], P3 [28], P4 [42], P7 [30], P8 [44], left [29] and right [47] mastoids, and two electrodes [62, 63] placed directly below the eyes. The EEG signal was amplified with a NetAmps300 amplifier. The online reference was Cz, and data were low-pass filtered at the Nyquist frequency (i.e., 100 Hz) for the sampling rate of 250 Hz. Impedances were kept below 100 kΩ.

EEG data processing

To monitor attention to the screen during the task, a video camera was placed above the computer screen focusing on the face of the child. Segments in which the child did not look at the screen were marked and not included in the EEG analyses. Participants who saw less than 50% of the social judgments (< 30 trials) were excluded from further analysis. After applying a .3 Hz high-pass filter (99.9% pass-band gain, .1% stopband gain, 1.5 Hz roll-off) EEG data were exported for further processing with Brain Vision Analyzer (BVA) 2.0 software (Brain Products, Inc). Offline, the EEG signal was filtered with a 30 Hz low-pass filter (–3 dB, 48 dB/octave). The event of interest was the presentation of the social judgment (2000 ms). Segments extending from 1000 ms before stimulus onset until 2500 ms after stimulus onset were extracted from the data. Segments containing artifacts were automatically rejected if the difference between the minimum and maximum voltage exceeded 300 μV within the –500–2000 ms interval around stimulus onset in any channel of the subset (see 2.4 EEG recordings) or was less than .5 μV activity within a 100 ms interval in any channel of the subset. Bad channels (i.e., channels in which artifacts occurred in over 50% of segments) were deleted from the dataset. Participants’ data was included in the analyses when at least 5 artifact-free trials (equal to 10 s) per condition were available (similar criteria have been used in studies of adults, see e.g. Harmon-Jones and Sigelman, 2001). On average 14 trials per condition were included (positive: M = 14 [range 5–20]; negative: M = 14 [range 6–20]; neutral: M = 14 [range 5–20]). A continuous wavelet transform (Morlet complex wavelet, 10 linear frequency steps from 2 to 20 Hz, morlet parameter c = 5, unit energy normalization) was used to calculate spectral power (μV2) within 10 frequency bands. We extracted the band with a central frequency of 8 Hz (bandwidth: 6.4–9.6 Hz) as a measure of alpha power (6–10 Hz in young children (Marshall et al., 2002)) for each trial and electrode. Average alpha power values within the 0–2000 ms interval were exported and natural log transformations were computed to normalize the data distributions. Frontal alpha asymmetry was computed by subtracting alpha activity over left frontal areas (electrode F3) from alpha activity over right frontal areas (electrode F4).

Data analysis

The behavioral data (mean pressing time per condition) and EEG data (frontal asymmetry) were checked for normality and outliers per sample. Pressing time showed one outlying value in the negative social judgment condition in test sample 1 (Z-value<−3.29) which was winsorized (Tabachnick and Fidell, 2006).

We used the MEMORE macro for SPSS (Montoya and Hayes, 2017) to examine whether frontal asymmetry during the SNAT-EC mediated the effect of condition (negative versus positive social judgments) on aggression (mean pressing time). Because the MEMORE macro allows for the inclusion of only two conditions in the within-subject mediation model, we decided to present results regarding the most important, likely largest, contrast of negative versus positive social judgments in the Results section and results regarding the other contrasts in the supplementary material. The MEMORE macro performs a series of regression analyses to estimate and test the effects of the independent variable, condition (negative vs. positive social judgments), on the mediator, frontal asymmetry (path a in Fig. 2) and on the dependent variable, pressing time (path c in Fig. 2). Also, the effect of the mediator on the dependent variable (path b) is tested. Finally, the overall mediation effect is tested by evaluating the significance of the indirect effect of the independent variable on the dependent variable through the mediator (path a * path b) using bootstrap analysis. The direct effect of the dependent variable on the independent variable that does not operate through the mediator is also computed (path c′ in Fig. 2). Due to the nature of the regression models used the average value of the mediator across conditions (i.e., average frontal asymmetry across positive and negative social judgments) is automatically included as a moderator in the model (see Montoya and Hayes, 2017 for a detailed explanation). Alpha was set to .05, and the significance of the indirect effect was tested using the percentile bootstrap method with 10,000 iterations.

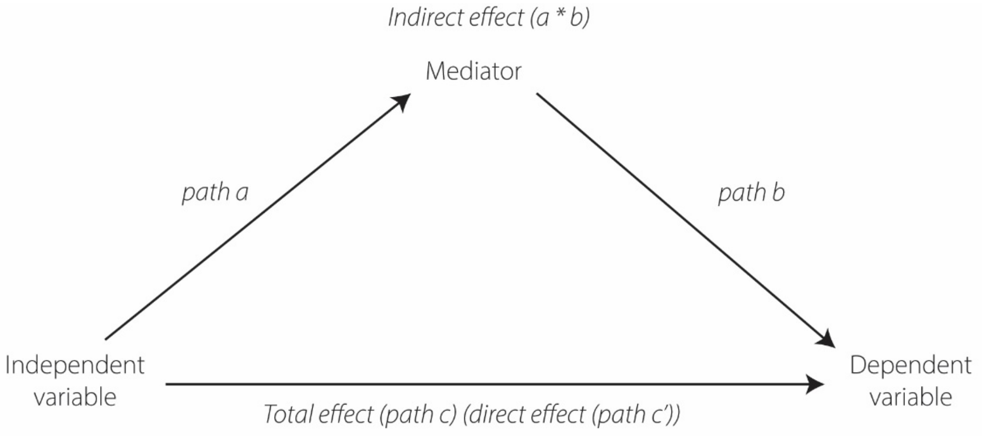

Figure 2. Within-subjects mediation model.

Finally, the results of the three samples were combined in a meta-analysis. Combined effect sizes were computed with the comprehensive meta-analysis (CMA) program using a random-effect model (Borenstein et al., 2009). We included t-values (with degrees of freedom) and standard errors in the meta-analysis to calculate Pearson correlations. To compute the effects of the mediation model the Pearson correlations were first transformed to Fisher z values and after meta-analytic combination back transformed to Pearson r's.

Results

Within-subjects mediation model

Results of the within-subject mediation models for all three samples are shown in Fig. 3. In the pilot sample a significant effect of condition (negative versus positive judgment) on aggression was found (total effect: b = 794.02, SE = 242.73, p<.01).[33] Negative judgments elicited on average 794 ms longer button presses than positive judgments, which corresponds to about two more balloons destroyed. This effect was not significantly mediated by frontal asymmetry in response to the social judgments (indirect effect: b = 9.32, bootstrapped SE = 79.88, 95% confidence interval (CI): −136.91 – 208.35), and the effect of condition on aggression remained significant when frontal asymmetry was taken into account (direct effect: b = 784.70, SE = 254.18, p<.01).

These effects were replicated in test sample 1: on average children pressed the button 802 ms longer (corresponding to two destroyed balloons) after a negative judgment compared to a positive judgment (total effect: b = 802.28, SE = 213.71, p<.01, direct effect: b = 853.87, SE = 219.64, p<.01). Again, this effect was not mediated by frontal asymmetry in response to the social judgments (indirect effect: b = −51.60, bootstrapped SE = 77.51, 95% CI: −234.44 – 81.96). Test sample 2 showed similar results: children pressed the button on average 828 ms longer (again corresponding to about two destroyed balloons) after negative judgments compared to positive judgments (total effect: b = 828.78, SE = 184.85, p<.01, direct effect: b = 861.54, SE = 176.50, p<.01), but this effect was not mediated by frontal asymmetry (indirect effect: b = −32.77, bootstrapped SE = 79.73, 95% CI: −192.32 – 142.38). Average frontal asymmetry across SNAT-EC conditions did not significantly moderate effects of condition in any of the three samples (pilot: b = 689.82, SE = 1145.20, p = .55, test 1: b = 842.56, SE = 970.52, p = .39 and test 2: b = −1064.87, SE = 963.73, p = .28).

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[3] The excluded sample (participants with behavioral data but no EEG data; pilot N = 18, test 1 N = 17, test 2 N = 16) showed similar effects and there were no significant differences between the included and excluded samples (pilot F(37) = .11, p = .74; test 1F(42)= .07, p = .80; test 2F(44) = 2.88, p = .09).

Meta-analysis

The results of the three samples were combined in a meta-analysis. The total effect of negative versus positive judgments on aggression showed a large combined effect size (r = .42, 95% CI: .29 – .54, p<.01). The indirect effect via frontal asymmetry was very small and not significant (r = −.03, 95%: −.13 −.07, p = .56). The direct effect of negative versus positive judgments on aggression controlled for effects on frontal asymmetry was similar to the total effect and significant (r = .34, 95% CI: .24 – .44, p<.01), see Table 2. All outcomes were homogenous (p>.05).

Table 2. Meta-analysis of the within-subjects mediation model effects on three samples

	Sample	r	95% CI	95% CI
Total effect	Pilot	.453**	.153	.676
	Test 1	.390**	.154	.585
	Test 2	.432**	.230	.598
	random effect	.422**	.290	.539
Path A	Pilot	.037	-.161	.231
	Test 1	.011	-.163	.184
	Test 2	-.038	-.201	.128
	random effect	-.001	-.104	.102
Path B	Pilot	.041	-.156	.236
	Test 1	-.091	-.261	.085
	Test 2	.182*	.014	.340
	random effect	.046	-.116	.206
Direct effect	Pilot	.299**	.477	.098
	Test 1	.328**	.484	.153
	Test 2	.383**	.526	.218
	random effect	.341**	.435	.240
Indirect effect	Pilot	.012	.208	-.185
	Test 1	-.059	.116	-.231
	Test 2	-.035	.131	-.199
	random effect	-.031	.072	-.133

* $p < .05$; ** $p < .01$

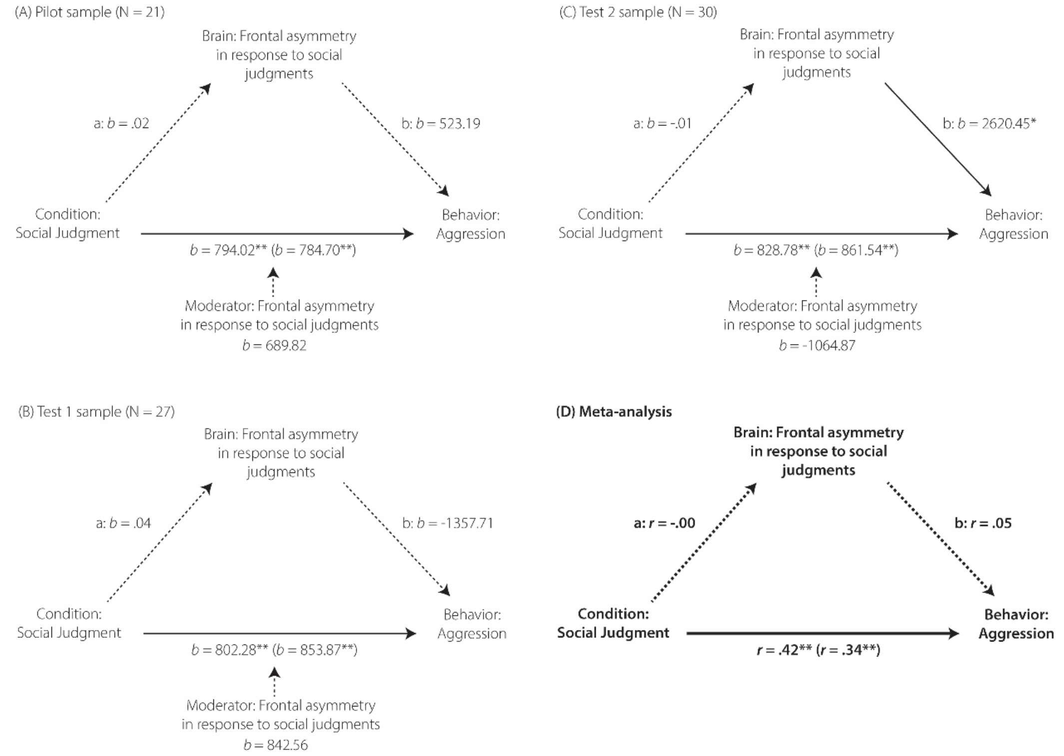

*Figure 3***.** Within-subjects mediation models shown separately for (A) pilot sample, (B) test sample 1 and (C) test sample 1. Combined results from the meta-analysis are shown in (D). A significant effect of social judgments (negative and positive) on aggressive behavior (mean pressing time) was found, shown with solid lines (** $p < .01$). This relation was not mediated by frontal asymmetry (solid line, * $p < .05$, dotted lines, $p > .05$). The indirect effect was not significant (pilot sample: $b = 9.32$, 95% CI: -136.91 - 208.35; test sample 1: $b = -51.60$, 95% CI: -234.44 - 81.96; test sample 2: $b = -32.77$, 95% CI: -192.32 - 142.38, meta-analysis: $r = -.03$, $p = .56$). Meta-analysis revealed a large combined effect size for the total and direct effect (solid line, ** $p < .01$).

Discussion

We investigated whether left frontal asymmetry mediates the relation between negative social judgments and aggressive behavior in young children. We included three samples (pilot, test 1 and test 2) to test the robustness of the results and combined our findings using meta-analysis. The results revealed a strong effect of social judgments on behavior: a negative social judgment led to more aggressive behavior than a positive social judgment. However, this effect was not mediated by frontal asymmetry. These results were replicated in all samples and a meta-analysis showed that the effect of social judgment on aggressive behavior is large.

A strong effect of social judgment on aggressive behavior conforms to our expectations and is in line with previous research showing a comparable effect of social judgment on aggressive behavior in adults (Achterberg et al., 2016) and older children (Achterberg et al., 2017; Overgaauw et al., submitted for publication; Dodge et al., 2003). The task design used in the current study was an adapted version of the SNAT used in the study by Achterberg et al. (2016, 2017) and Overgaauw et al. (submitted for publication) in which participants could respond to the judging peer with a loud noise blast. By replicating these behavioral results in young children, we have shown that the SNAT-EC is an age appropriate task to examine the behavioral response to social judgments in early childhood. Moreover, the meta-analysis for positive versus negative social judgments revealed a large combined effect size, providing evidence that negative social judgments indeed result in more aggressive behavior. Furthermore, the effect was replicated in two samples which together with the large meta-analytic results indicates that the effects found are robust. However, we do see smaller effect sizes for the effect of social judgments on aggressive behavior in early childhood (ω2 ranging from .12 to .15) compared to 7–10 year old children (ω2 ranging from .30 to .46; Achterberg et al., 2017) and adults (ω2 = .41; Achterberg et al., 2016). An important issue for further research is whether the increasing effect of social judgments on aggressive behavior as measured with the SNAT is related to the more profound emotional impact of rejection or with improving cognitive and/or motor skills over age.

Contrary to our expectations, left frontal asymmetry did not mediate the relation between negative social judgments and aggressive behavior. Our mediation hypothesis was based on studies showing a relation between greater relative left frontal activity and anger and aggression in adults (Harmon-Jones, 2004; Harmon-Jones and Sigelman, 2001; Verona et al., 2009). Results obtained with adults may not be directly generalizable to children both because of potential developmental issues and because of differences related to the behavioral and neural measures obtained (including e.g., data quality and quantity, and the selection of EEG frequency bands). We relied on evidence from adult samples, because studies relating frontal asymmetry to direct measures of aggressive behavior in children were lacking. Instead, aggressive behavior in children is often examined using parent and teacher reports. Indeed, only few studies have investigated relations between frontal asymmetry and caregiver-reported externalizing behavior (which includes, but extends beyond aggressive behavior) in young children. A recent meta-analysis showed no relation between left frontal asymmetry and externalizing behavior (effect size of d = .04, p = .79; (Peltola et al., 2014)). Although this, in combination with our own findings, suggests that left frontal asymmetry may not be related to aggressive behavior in early childhood, some caveats regarding the quantification of cortical activity in early childhood must be kept in mind. First, frontal asymmetry studies in children often do not report the minimum number of trials used in their analyses, neither for resting/trait-related frontal asymmetry nor state-related frontal asymmetry. Future research should investigate the reliability of frontal asymmetry in children in order to determine the minimum amount of EEG-data needed for reliable frontal asymmetry scores. Second, the frequency composition of the EEG is known to change over the course of development, but whether and how this affects frontal asymmetry is poorly understood (Saby and Marshall, 2012). Although research has directly related power in the 8–12 Hz EEG alpha band in adults to deactivation of cortical tissue (Cook et al., 1998; Laufs et al., 2003), no such evidence is, to the best of our knowledge, available for young children. Rather, estimates of the alpha frequency bandwidth in infants and young children are based on developmental changes in the peak frequency of the EEG (Marshall et al., 2002). Previous studies have varied in their choice of the alpha bandwidth (see Peltola et al., 2014), limiting the possibility to compare our results to previous findings. Studies examining the development of the EEG frequency composition, 'alpha' bandwidth, and frontal asymmetry in children are thus badly needed.

In addition, we chose to focus on frontal asymmetry because of its suggested link to aggressive feelings and behaviors (expressed in destroying balloons in the SNAT-EC), but primary emotional responses to rejection, preceding aggression, may also be of relevance. Some children might feel sad after receiving a negative social judgment whereas others might feel angry. Both emotions can lead to aggressive behavior (see e.g. Buckley et al., 2004), but they may impact differently on patterns of frontal asymmetry, as sadness, in contrast to anger, is a withdrawal-related emotion (Coan et al., 2001). Future studies should additionally measure participants' (primary) emotional responses to positive, negative and neutral social judgments. However, it is important to note that the children in the current study were relatively young and might therefore experience problems in correctly indicating or nuance their emotional state (Chambers, and Johnston, 2002).

Future studies could also address some limitations of the current study. First, the external validity of laboratory measures is sometimes debated: it is questioned whether findings obtained using experimental tasks in laboratory settings generalize to real life situations. However, aggression measured in a laboratory setting was meta-analytically shown to be highly generalizable to real-world aggression (Anderson and Bushman, 1997). In addition, an observational study by Dodge et al. (2003) investigating social rejection and aggression showed similar findings to our own: social rejection by peers was related to an increase in aggressive behavior. We therefore feel that the conclusion that our experimental paradigm (SNAT-EC) is relevant for examining aggressive behavior in response to social judgments in early childhood is warranted. Second, the three samples were relatively small, mainly because about 50% of the participants in each sample provided no usable data. Such percentages of attrition are, however, rather common in EEG research with young children (Bell and Cuevas, 2012). It is quite challenging for children to sit still during EEG measurements (resulting in relatively high percentages of movement and ocular artefacts) and some children refuse to wear a cap or net at all. Although the sample sizes are relatively small, note that the results of the pilot sample were replicated in the two other samples, and that the meta-analysis showed a large effect size for the relation between social judgment and aggressive behavior, enhancing confidence in the validity and robustness of our findings. Furthermore, as the power to detect an indirect effect is as large as or (often) larger than the power to detect the main and direct effects, power and sample size are not of greater concern for mediation analysis (Kenny, and Judd, 2014). Next to that, we used a within-subjects design which has increased statistical power compared to a between-subjects design as it doesn’t include error variance due to stable individual differences (Kenny, and Judd, 2014). Nevertheless, adequate sample size remains an important consideration for future studies and we continue to search for ways to enhance children's willingness to comply with EEG measurements. Third, one could argue that, children may like to destroy balloons and that, as a consequence, we were not measuring aggressive behavior in response to social judgments. However, we controlled for individual differences in children's pleasure or interest in popping balloons by using a within-subjects design, in which we compared the mean pressing time after negative social judgments to the mean pressing time after positive social judgments on an individual level. Finally, we decided not to judge the children on personal characteristics but on a self-chosen cuddly animal for ethical reasons, which might have influenced the results. However, as stated above, the behavioral results were very robust. In addition, the children had been playing with the cuddly animal in the two weeks prior to the lab visit and they were clearly attached to their cuddly animal as evident from the stories the children told us, many children gave the cuddly animal a name and carried it along everywhere they went. Thus, we are confident that our paradigm successfully elicits experiences of (mild) rejection. For a measure of the children's attachment to the cuddly animal, future research may include questions asking for example how much time the child spent with the animal and whether it was the child's favorite toy.

Future studies should search for other neurocognitive mechanisms that may mediate the relation between social judgments and aggressive behavior. One might think of several event related potential (ERP) components as possible mediators, for example, components related to the processing of negative feedback, like the FRN (Feedback-Related Negativity) or components reflecting the allocation of attention like the P3 (Luck, 2014). A study in adults using the social judgment paradigm by Somerville and colleagues (2006) found an enhanced P3 only after expected acceptance (van der Veen et al., 2013). However, another study in adults did not find significant differences between positive and negative social judgments in FRN or P3 amplitudes (van der Molen et al., 2014). These authors did, however, find increases in midfrontal theta power, believed to index feedback processing, after unexpected rejection (van der Molen et al., 2016). In the current study we could not test the mediating role of ERPs, because the reliable measurement of ERP components requires larger numbers of artifact-free trials than were available from our participants (see also Huffmeijer et al., 2014). Theta power warrants study as a possible mediator. However, more research on the development of the theta frequency band is necessary (Saby and Marshall, 2012).

In conclusion, the current study showed that the SNAT-EC is an age appropriate task to reliably measure aggressive behavior in response to negative social judgments in young children. Frontal asymmetry during the task did not mediate the relation between social judgment and aggressive behavior in early childhood and other neurocognitive mechanisms should be examined to bridge the brain-behavior gap between social judgments and aggressive behavior.

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CHAPTER 5

General discussion

General discussion

The general aim of this thesis was to gain more insight in the neural correlates of temperament and social behavior in early childhood. We were specifically interested in frontal asymmetry (FA) in relation to fearfulness, prosocial behavior and aggressive behavior. Our results, described in Chapter 2, showed that individual differences in both fearfulness and FA are best explained by a combination of genetic influences (about a quarter of the variance) and unique environmental influences (about three quarters of the variance). In contrast to our expectations these influences on fearfulness and FA were not overlapping which indicates that on the level of developmental contributors our data do not support an association between fearfulness and FA in early childhood.

In Chapter 3 and Chapter 4 we examined two new tasks to measure social behavior in reaction to social exclusion and social judgments. The results in Chapter 3 consistently showed that the vast majority of the children compensated for social exclusion in the first trials of the Prosocial Owl Game (POG). Individual differences in prosocial behavior arose when the game was progressing. Similar to the Prosocial Cyberball Game for older children and adults (Riem, Bakermans-Kranenburg, Huffmeijer, & Van IJzendoorn, 2013; Vrijhof et al., 2016; Van der Meulen, Van IJzendoorn, & Crone, 2016; Van der Meulen et al., 2017) this newly developed POG successfully triggered prosocial compensating behavior. We hypothesized that individual differences would be explained by differences in approach and withdrawal tendencies as reflected by FA, however this was not the case.

In Chapter 4 we examined how children responded to social judgments from peers on their cuddly animal. We successfully adapted the Social Network Aggression Task (SNAT) used in older children and adults (Achterberg, Van Duijvenvoorde, Bakermans-Kranenburg, & Crone, 2016; Achterberg et al., 2017) and made an ethically accepted version for Early Childhood (SNAT-EC). In line with the SNAT for older children and adults we showed that young children responded in a more aggressive way after a negative social judgment than after a positive social judgment during the SNAT-EC. Contrary to our expectations, this reaction was not mediated by FA. In other words, negative social judgments did not induce relatively greater left frontal activity and left FA was not related to more aggressive behavior. In sum, the results of our newly developed tasks for social behavior in early childhood showed similar results of prosocial compensating behavior and aggressive behavior in response to social feedback in children as in adolescents and adults. However, FA was not related to this behavior.

In the following sections we will elaborate on our twin design, on the replicability of our findings, and on possible explanations for our results regarding FA. Next we will discuss the limitations and some directions for future research.

Behavioral genetics and replicability

All studies presented in this thesis included twin samples. Twin samples create great research opportunities for two distinct aims: examining heritability and testing the replicability of results. Using genetic modelling we investigated genetic and environmental influences on the traits. We examined the monozygotic (MZ)/dizygotic (DZ) correlations to estimate heritability as higher MZ correlations than DZ correlations suggest genetic influences on the trait. In addition, twin samples can be used for a test-replication design. During the past years the importance of replicability in research has been highlighted (see Pashler & Wagenmakers, 2012). Especially when examining new tasks in a study, it is important to replicate the outcomes of the study to enhance confidence in the validity and robustness of the findings. In this thesis we examined two newly developed tasks to measure prosocial and aggressive behavior in early childhood and we did so using a test-replication design. Two similar groups were created in which each child from a twin pair was randomly assigned to either the test or the replication sample.

Behavioral Genetics. This thesis showed that fearfulness, FA and prosocial compensating behavior are partly influenced by genetic factors. In Chapter 2 we used bivariate genetic modelling and showed that both fearfulness and FA were best explained by unique environmental influences (for about three quarters) and also for a part by genetic influences (for about one quarter). Our results were in line with previous studies (Anokhin, Heath, & Myers, 2006; Clifford, Lemery-Chalfant, & Goldsmith, 2015; Goldsmith, Buss, & Lemery, 1997; Lemery-Chalfant, Doelger, & Goldsmith, 2008; Smit, Posthuma, Boomsma, & De Geus, 2007; Van Houtem, Laine, Boomsma, Ligthart, Van Wijk, & De Jongh, 2013). However, in general these studies found larger genetic influences. The relative contribution of unique environmental versus genetic influences to personality traits seems to change over the lifespan and during childhood genetic influences seem to increase over the years (see a meta-analysis by Kandler & Papendick, 2017). Thus, developmental changes may explain the relatively low influence of genetics on fearfulness and FA in our study compared to other studies that examined 8-year-old children (Clifford et al., 2015; Lemery-Chalfant et al., 2008). In Chapter 3 we examined the MZ/DZ correlations to estimate the heritability of prosocial compensating behavior. As there was almost no variance in the first trials of the game (because most children compensated for exclusion) we estimated heritability over the second and third trials. In accordance with previous studies (see review by Knafo-Noam, Vertsberger, & Israel, 2018) we showed a large heritability component for prosocial compensating behavior.

Overall, we found that genetic factors are involved in fearfulness, FA and prosocial behavior, which indicates that some of the negative results in this thesis do not merely reflect measurement error as the substantial correlations between children within MZ twin pairs would not emerge when only non-systematic error was at stake. However, we cannot ignore the fact that we also found substantial influence of unique environmental influences that include measurement error. Recently a longitudinal cross-cultural study showed that most variation in behavioral and emotional adjustments (based on self-reports of 8-12 year old children) was explained by within-person variability, rather than between-person or between-group variability (Deater-Deckard et al., 2018). The authors interpreted this within-person variability as measurement error (K. Deater-Deckard, personal communication, May 23, 2018).

Accordingly, we believe that measurement error also plays a role when collecting data using tests and observations.

Replicability. We developed two tasks to measure social behavior in response to social exclusion and social judgments in early childhood and used a test-replication design to examine the replicability of our outcomes. In Chapter 3 we investigated the POG and showed that prosocial compensating outcomes were comparable in the test and replication sample. In line with previous studies (Van der Meulen et al., 2016, Vrijhof et al., 2016) we found that children show prosocial compensating behavior during the POG. Especially during the first trails of each game most children compensated. Individual differences arose in the second and third trials of the game. We hypothesized that children that compensated more over all trials would also have higher ratings of prosocial behavior as reported by the parents or would donate more stickers to an unknown child. However, in both the test and the replication sample we found that the different indicators for prosocial behavior were not related. Other studies also failed to find associations between different prosocial responses in infants (Dunfield, Kuhlmeier, O'Connell, & Kelley, 2011; for a review see Thompson & Newton, 2013), adolescents (Vrijhof et al., 2016) and adults (Van der Meulen et al., 2016). Indeed, prosocial behavior has been suggested to be a multidimensional construct for which outcomes are dependent on context and the type of prosocial behavior measure (Paulus, 2018; Padilla-Walker & Carlo, 2015).

In Chapter 4 we investigated the SNAT-EC and validated our findings on aggressive behavior in response to negative social judgments in three samples: a pilot sample in which we tested our hypotheses and a test and replication sample in which we showed that the outcomes were replicated. By using a meta-analysis over the three samples we found a large effect size of social judgment on aggressive behavior. Thus, in line with previous studies in older children and adults (Achterberg et al., 2016, 2017) we showed that negative social judgments induce more aggressive behavior than positive social judgments. Contrary to results of studies in adults which consistently showed that aggressive behavior is related to left FA (see a recent review by Harmon-Jones & Gable, 2018), we did not find a relation between FA and aggressive behavior in early childhood.

Frontal asymmetry in early childhood

Contrary to our expectations, FA was not associated with fearfulness, prosocial behavior or aggressive behavior in early childhood. In Chapter 2 we hypothesized that fearfulness would be associated with relatively greater right frontal brain activity (or right FA), but our results showed no significant correlations. In addition, there was no overlap in genetic or environmental influences that explained the variance in both fearfulness and FA. Studies reporting associations between right FA and withdrawal behavior mainly focused on clinical samples with depression and anxiety problems (see meta-analysis by Thibodeau, Jorgensen, & Kim, 2006). Even though anxiety symptoms have been associated to fearfulness (Goldsmith & Lemery, 2000), it could be that a significant association between fearfulness and right FA is only present in populations with more severe fear/anxiety problems. In our study we included typically developing children and previous studies including similar samples also failed to show significant relations between fearfulness and FA (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Howarth, Fettig, Curby, & Bell, 2016). In addition, a study including adults that distinguished between fear and anxiety only found an association between right FA and anxiety, not fear (Neal & Gable, 2017). To conclude, having a non-clinical sample with young children may explain why we did not find a significant association between FA and fearfulness.

In this thesis we also investigated the relation between FA and approach-related behavior. We hypothesized that prosocial compensating behavior would be related to relatively greater left frontal brain activity (left FA). We based our hypotheses on studies that investigated prosocial behavior in infants (helping and comforting behavior; Paulus, Kühn-Popp, Licata, Sodian, & Meinhardt, 2013) or in adults (donating behavior; Huffmeijer, Alink, Tops, Bakermans-Kranenburg, & Van IJzendoorn, 2012). In addition, as described in the previous section, prosocial behavior has been suggested to be a multidimensional construct (Paulus, 2018; Padilla-Walker & Carlo, 2015) which might also explain why we did not find a relation between

FA and prosocial compensating behavior. Thus, previous studies on which we based our hypotheses are in retrospect of limited value because they were carried out in other age groups or focused on other forms of prosocial behavior.

Furthermore, we hypothesized that aggressive behavior in early childhood would be associated with left FA. Even though there are studies investigating aggressive behavior in response to social judgments in early childhood (e.g. Dodge et al., 2003; Buckley, Winkel, & Leary, 2004) there is a lack of studies that relate this behavior to FA in children. On the other hand, there are a lot of studies that associate aggressive behavior to left FA in adults (see a recent review by Harmon-Jones & Gable, 2018). Still, our study did not find a relation between aggressive behavior in response to social judgments and FA in early childhood. There are several explanations for our results. Overall our hypotheses were mainly based on adults or infant studies examining FA (Thibodeau et al., 2006; Harmon-Jones & Gable, 2018; Paulus et al., 2013; Huffmeijer et al., 2012; Coan & Allen, 2004). The main issue is that results obtained with adults may not be directly generalizable to children.

First, potential developmental issues arise because of differences related to behavioral and neural measures obtained. Adults, of course, tend to provide much more and much 'cleaner' data than young children. Obtaining EEG data from children is challenging; the attention span of children is much shorter and long measurements lead to more rather than less artefacts (movements, eye blinks, changes in behavioral state like drowsiness; see Bell & Cuevas, 2012). In order to reduce these artefacts we adjusted the tasks for children in order to keep them motivated. For example, we included extra breaks during the SNAT-EC. Furthermore, as described in the discussion of Chapter 4, the frequency composition of the EEG is known to change over the course of development, but whether and how this affects FA is poorly understood (Saby & Marshall, 2012). Alpha band frequencies in children are based on developmental changes in the peak frequency of EEG (Marshall, Bar-Haim, & Fox, 2002). However, it is unknown whether the alpha frequency range of 6 – 10 Hz indeed corresponds to other measures of cortical tissue deactivation because this has not been examined in children. Future research should examine the development of the EEG frequency composition, ‘alpha’ bandwidth, and FA in children.

Second, there is still much debate about the minimum amount of EEG data needed for a reliable FA measurement. In our studies the number of trials used to compute FA is relatively small compared to adults studies. Based on the study by Tomarken and colleagues (1992) most researchers suggest that 8 minutes of resting EEG is necessary to obtain reliable FA (Tomarken, Davidson, Wheeler, & Kinney, 1992). However, a review by Allen and colleagues (2004) showed that shorter time frames are not less reliable (Allen, Coan, & Nazarian, 2004). Because children have more difficulty to remain calm and concentrated during the measurements it is necessary to compromise between the quality and quantity of the EEG data in early childhood. A study examining the development of EEG from 5 months till 4 years of age (Marshall et al., 2002) used a EEG rest measurement of less than 80 seconds to compute FA in 4 year old children. They used a mean number of artifact-free segments of 67 seconds (SD = 20); in our studies on average 63 segments per condition were included, leading to an average of somewhat more than 120 seconds in total (as we had two conditions for rest FA: eyes open and eyes closed). We therefore believe that our EEG rest measurement of three minutes with on average 63 segments per condition is sufficient to compute reliable FA. In Chapter 4 we based our minimum amount of trials on an adult study by Harmon-Jones & Sigelman (2001); they used a minimum criterion of 10 artifact-free seconds, which is similar to our minimum of 5 trials (equal to 10 seconds). Nevertheless, it is important for future studies to determine the optimal number of trials needed for reliable FA data in children.

Furthermore, in this thesis we used both so-called trait-related (measured during rest) and state-related (measured during a task) FA scores. In Chapter 2 we examined FA measured during rest in relation to parent-reported fearfulness rather than fear-inducing stimuli because we were interested in more stable, task-independent, traits. Other studies using parent-reported fearfulness and trait-related FA in children also failed to find significant relations (Diaz & Bell., 2012; LoBue, Coan, Thrasher, & DeLoache, 2011) or suggest more complex relationships. For trait-related FA the question may arise to what extent FA actually reflects a stable trait, as discussed more thoroughly in Chapter 2. It has been suggested that state-related fluctuations are also present during a rest measurement and as a result the maximum genetic influence on individual differences in FA cannot exceed 60% (Hagemann, Naumann, Thayer, & Bartussek, 2002; Smit et al., 2007). This may explain why we found large unique environmental influences on FA and somewhat lower genetic influences. However, even though our data showed only low heritability, it also shows that our data is not only driven by measurement error alone (which is included in the unique environmental influences).

Finally, following Chapter 2 and 3 in which our results suggest that FA (measured during rest) is only related to temperament or behavior in a more complex way we examined FA in Chapter 4 differently. Coan and Allen (2004) reviewed FA studies and proposed that FA should be studied as a moderator or mediator of emotion- and motivation related construct, such as aggression. We therefore collected state-related FA measured during the SNAT-EC and hypothesized that aggressive behavior after a negative social judgment would be mediated by greater left frontal activity. Nevertheless, our results did not support this hypothesis; a negative social judgment was not associated with relatively greater left frontal brain activity and left FA was not related to more aggressive behavior. This is especially surprising as a recent review suggests that individual differences in FA are more pronounced when an emotion is evoked than at rest (Reznik & Allen, 2018). However, possibly developmental issues like the EEG frequency composition, alpha bandwith determination or the minimum amount of EEG data to compute FA as described above may be involved when examining the mediating role of state-related FA in early childhood.

Limitations & future directions

The studies presented in this thesis have some limitations that should be addressed in future research. First, the studies described in this thesis were part of the larger, longitudinal L-CID study with a broader focus than just the EEG measurement. Several other measurements were included as well, both individual tasks as well as parent-child interactions tasks, resulting in a duration of approximately 3 hours per lab visit. Each co-twin was randomly assigned to the EEG block or the behavioral block as their first task. It is quite challenging for children this age to sit still during EEG measurements, and in combination with tiredness from the other tasks, this may have caused an increase in movement and ocular artifacts in the EEG data. About 33-50% of the data was unavailable because of artifacts, technical problems or refusal, however, such attrition rates are common in early childhood (see Bell & Cuevas, 2012). Besides, our sample was quite large compared to other EEG-studies with children, resulting in sufficient EEG-data for the analysis.

Secondly, all data used in this thesis were measured at one time point. Recent FA research has shown the importance of longitudinal data as studies have shown that some associations emerge over a longer time period (for instance see Goldstein et al., 2018). In addition, causality and direction can only be examined in longitudinal designs. Longitudinal behavioral data with two time points for the EEG data will be available in the future within the L-CID project, but for the current thesis such data were unavailable.

Further, the generalizability of findings from twin research to singletons may be questioned. However, research has shown that singletons and twins do not differ on temperament (Goldsmith & Campos, 1990), personality (Johnson, Krueger, Bouchard, & McGue, 2002) or externalizing behavior (Robbers et al., 2010) which suggests that twins and singletons are not so different from each other after all. Still, with regard to internalizing problems Robbers and colleagues (2010) suggest that twins may help each other against developing internalizing problems in early adolescence. Whether such protective factors for twins compared to singletons are already present in early childhood is unknown and future research should investigate the differences between twins and singletons more thoroughly.

Concluding remarks

In the current thesis we examined FA in relation to fearfulness, prosocial behavior and aggressive behavior. Based on the literature asymmetric frontal brain activity was a likely candidate to explain individual differences in approach and withdrawal related behavior in young children. However, our results showed no associations between fearfulness, prosocial behavior or aggressive behavior and FA in 4-6 year old children. We did show that genetic influences were involved in fearfulness and prosocial behavior in 4-6 year old children (see Chapter 2). Furthermore, in this thesis we presented two new tasks (POG and SNAT-EC) with which we showed that young children show similar increases in prosocial behavior in response to social exclusion and aggressive behavior in response to social judgments as older children, adolescents and adults, with comparable effect sizes (see Chapter 3 and 4). We showed that the POG and SNAT-EC are reliable measures and may conclude that the studies in this thesis can be used as a basis for follow-up research. In combination with the Prosocial Cyberball Game (PCG; Riem et al., 2013; Vrijhof et al., 2016; Van der Meulen, et al., 2016; 2017) and the SNAT (Achterberg et al., 2016, 2017) that measure the same social constructs in older children, adolescents and adults, the POG and SNAT-EC make it possible to use the tasks in longitudinal designs from early childhood to adulthood. The data collected for this thesis will be of increasing value in the coming years in which longitudinal data will be collected to investigate temperament and social behavior in children aged 4-13 years old (see Euser et al., 2016). The role of FA remains unclear but new insights may be revealed when the children grow older.

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CHAPTER 6

Supplementary Material

Supplemental material

Chapter 2

Univariate models for Fear, EC, and FA and additional bivariate model Fear and FA

Here we present the results of the univariate behavioral genetic models and the bivariate behavioral genetic model for Fear and FA only, including the participants with complete data for both variables.

Fear. The results of the univariate model with Fear showed that the AE model has the best fit, $\Delta\chi^2$ (3) < 3.84, p > .05. Path loadings revealed that variation in Fear is explained by genetic factors (35%) and by unique environmental factors (65%), which is quite similar to the path loadings for Fear in the bivariate models.

Effortful control. For EC, the univariate model showed that AE has the best fit, $\Delta\chi^2$ (3) < 3.84, p > .05. Path loadings indicated that the individual differences in EC are explained by genetic factors (26%) and by unique environmental factors (74%). This ratio between AE is highly comparable to path loadings for EC in the bivariate models.

Frontal asymmetry. The results of the univariate model with FA showed that AE and CE are both significantly better than ACE ($\Delta\chi^2$(3) < 3.84; p > .05). However, the CE model has a slightly lower AIC value (AIC = -571.26), than the AE model (AIC = -570.58) meaning that shared and unique environmental factors can best explain individual differences in FA. Path loadings showed that individual differences in FA are explained by shared environmental factors (23%) and by unique environmental factors (77%). This is comparable to the path loadings of unique environmental factors of FA in the bivariate models.

Fear and Frontal asymmetry. The bivariate model with Fear and FA containing only participants with complete data on Fear and FA (n = 107 with 67 MZ and 40 DZ twin pairs) also shows that the AE model had the best fit, $\Delta\chi^2 < 3.84$, $p > .05$, showing that genetic and unique environmental factors account for the variation in Fear and FA. The path loadings show that individual differences in Fear were explained by genetic factors (38%) and unique environmental factors (62%). Variation in FA was explained by genetic factors (19%) and unique environmental factors (81%). These path loadings are similar to the bivariate model with Fear and FA containing imputed FA data, indicating that FIML modeling estimated the missing data correctly.

Supplemental material

Chapter 3

Table 1. Items from MCQ and SDQ questionnaires with PCA factor loadings

			Factor loadings			
			test sample		replication sample	
Factor	Items	Question-naire	PP	OP	PP	OP
1 Empathy	Likely to ask, "What's wrong?" when seeing someone in distress	MCQ	.71	.64	.62	.58
	Often volunteers to help others	SDQ	.69	.73	.58	.65
	Can tell at just a glance how others are feeling	MCQ	.69	.68	.55	.56
	Helpful if someone is hurt	SDQ	.67	.72	.81	.72
	Considerate of other people's feelings	SDQ	.66	.69	.71	.69
	Will try to comfort or reassure another in distress	MCQ	.59	.68	.64	.68
	Will feel sorry for other people who are hurt, sick, or unhappy	MCQ	.57	.57	.31	.26
	Shares readily with other children	SDQ	.55	.59	.58	.67
	Likely to offer toys or candy to a crying playmate even without parental suggestion	MCQ	.45	.75	.42	.54
	Likely to show spontaneous nurturing and care-giving behavior toward an animal	MCQ	.38	.32	.34	.56
	Kind to younger children	SDQ	.23	.11	.58	.33
2 Contagion	Is upset by stories in which characters are hurt or die	MCQ	.83	.74	.86	.86
	Gets angry at aggressor, "Bad Guy", who hurts a TV character	MCQ	.64	.63	.75	.64
	Acts upset when she or he sees a hurt animal	MCQ	.57	.56	.52	.72
	Is not likely to become upset if a playmate cries.	MCQ	.54	.43	.28	.03

Note: PP = primary parent; OP = other parent. Factor loadings from rotated component matrix (Varimax with Kaiser rotation).

Supplemental material

Chapter 4

Here we present the results of the mediation models including the contrasts neutral versus positive social judgments and negative versus neutral social judgments.

Within-subjects mediation model – neutral versus positive social judgments

The within-subjects mediation model for neutral versus positive social judgments showed a significant effect of condition on aggression in the pilot sample (total effect: b = 410.08, SE = 145.45, p = .01). On average neutral social judgments elicited 410 ms longer button presses than positive social judgments, which corresponds to about 1 more destroyed balloon. This effect was not significantly mediated by frontal asymmetry (indirect effect: b = -36.18, bootstrapped SE = 62.94, 95% confidence interval (CI): -164.15 – 96.23), and the effect of condition on aggression remained significant when taking frontal asymmetry into account (direct effect: b = 446.26, SE = 132.50, p < .01).

These effects were replicated in test sample 1: On average children pressed the button 382 ms longer after a neutral social judgment compared to a positive social judgment (total effect: b = 382.47, SE = 144.64, p = .01, direct effect: b = 380.32, SE = 149.82, p = .02). Again, this effect was not mediated by frontal asymmetry (indirect effect: b = 2.15, bootstrapped SE = 29.74, 95% CI: -71.30 – 56.46).

In test sample 2, the direct and total effect were marginally significant: children pressed the button on average 198 ms longer after neutral social judgments compared to positive social judgments (total effect: b = 197.70, SE = 103.00, p = .06, direct effect: b = 190.36, SE = 104.47, p = .08). Furthermore, this effect was not mediated by frontal asymmetry (indirect effect: b = 7.34, bootstrapped SE = 32.19, 95% CI: -66.34 – 68.19).

Average frontal asymmetry significantly moderated effects of condition on aggressive behavior in two of the three samples (pilot: b = -1128.93, SE =526.04, p = .05, test 1: b = 91.80, SE = 618.83, p = .88 and test 2: b = 1136.05, SE = 555.42, p = .05).

The total effect of neutral versus positive judgments on aggression showed a small to medium combined effect size (r = .17, 95% CI: .07 – .26, p < .01). The indirect effect via frontal asymmetry was very small and not significant (r = -.01, 95%: -.11 – .10, p = .91). The direct effect of negative versus positive judgments on aggression was similar to the total effect and significant (r = .22, 95% CI: .12 – .32, p < .01). All studies were homogenous (p > .05).

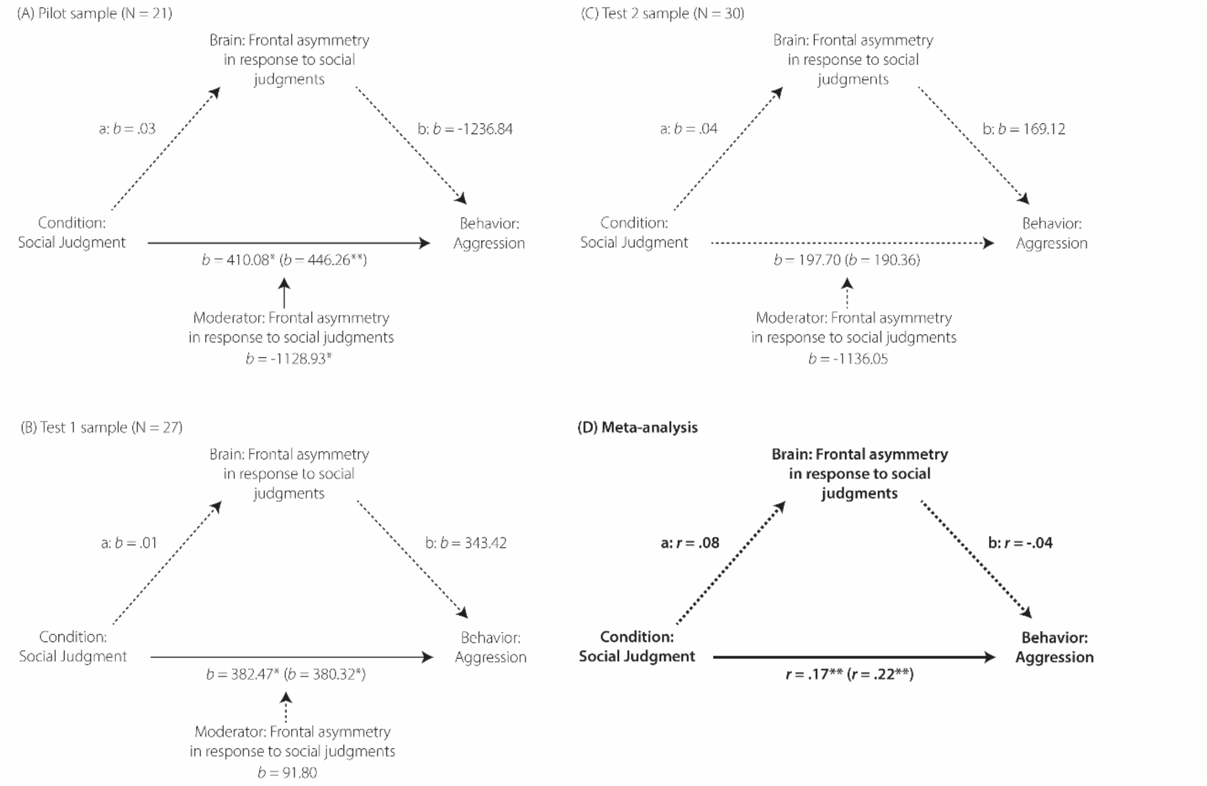
Within-subjects mediation model – negative versus neutral social judgments

Regarding the negative versus neutral judgments the within-subjects mediation model showed a marginally significant effect of condition (negative versus neutral) on aggression in the pilot sample (total effect: b = 383.95, SE = 186.73, p = .05). On average negative social judgments elicited 383 ms longer button presses than neutral social judgments, which corresponds to about 1 more destroyed balloon. This effect was not significantly mediated by frontal asymmetry (indirect effect: b = -11.93, bootstrapped SE = 57.88, 95% CI: -140.31 – 116.29), and the effect of condition on aggression remained marginally significant when taking frontal asymmetry into account (direct effect: b = 395.87, SE = 188.54, p =.05).

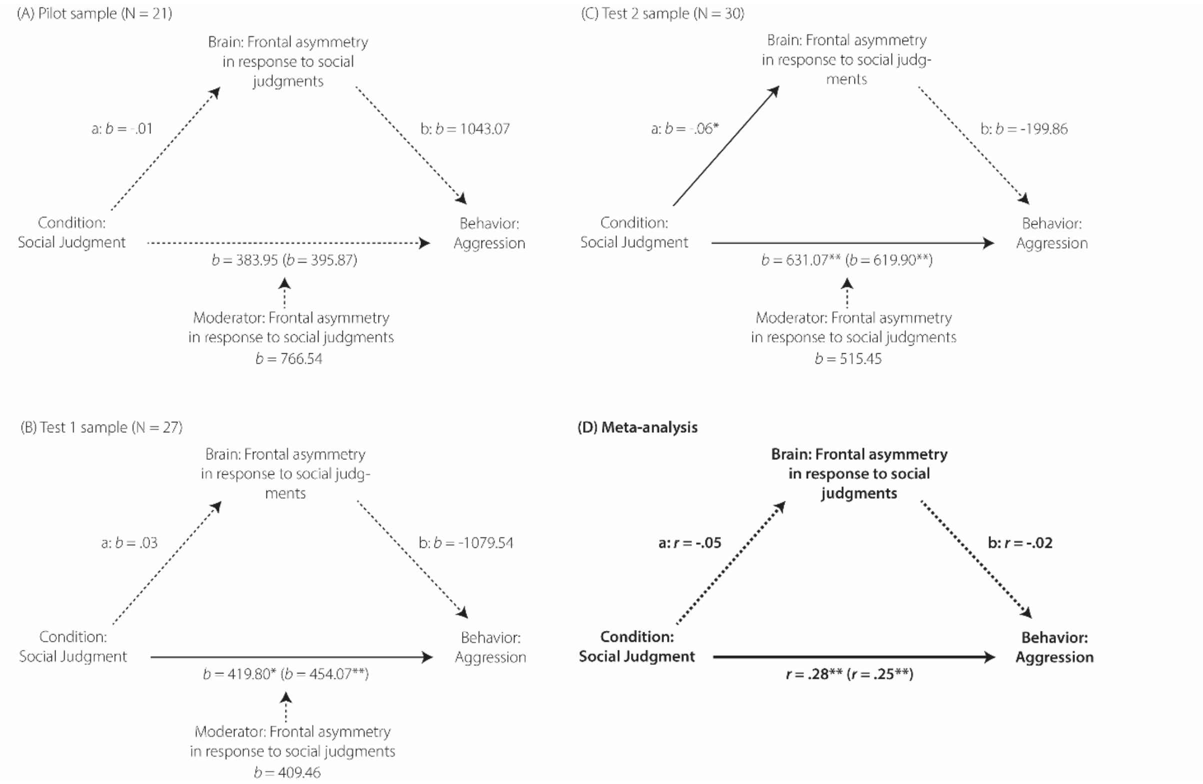
These effects were replicated in test sample 1: On average children pressed the button 420 ms longer after a negative social judgment compared to a neutral social judgment (total effect: b = 419.80, SE = 154.57, p = .01, direct effect: b = 454.07, SE = 153.30, p < .01). Again, this effect was not mediated by frontal asymmetry (indirect effect: b = -34.27, bootstrapped SE = 65.30, 95% CI: -214.30 – 37.02). In test sample 2 the children pressed the button on average 631 ms longer after negative judgments compared to neutral social judgments (total effect: b = 631.07, SE = 167.70, p < .01, direct effect: b = 619.90, SE = 185.34, p < .01), but this effect was not mediated by frontal asymmetry (indirect effect: b = 11.17, bootstrapped SE = 72.61, 95% CI: -141.19 – 163.93).

Average frontal asymmetry across SNAT-EC conditions did not significantly moderate effects of condition in any of the three samples (pilot: b = 766.54, SE = 890.93, p = .40, test 1: b = 409.46, SE = 667.40, p = .55 and test 2: b = 515.45, SE = 880.76, p = .56).

The total effect of negative versus neutral social judgments on aggression showed a small to medium combined effect size (r = .28, 95% CI: .16 – .39, p < .01). The indirect effect via frontal asymmetry was very small and not significant (r = -.02, 95%: -.12 –.09, p = .75). The direct effect of negative versus neutral social judgments on aggression was similar to the total effect and significant (r = .25, 95% CI: .15 – .35, p < .01). All studies were homogenous (p > .05).



Supplementary Material Figure 1. Within-subjects mediation models shown separately for (A) pilot sample, (B) test sample 1 and (C) test sample 1. Combined results from the meta-analysis are shown in (D). Significant effects of social judgments (neutral and positive) on aggressive behavior (mean pressing time) are shown with solid lines (** $p < .01$, * $p < .05$). This relation was not mediated by frontal asymmetry (dotted lines, $p > .05$). The indirect effect was not significant (pilot sample: b = -36.18, 95% CI: -164.16 – 96.23; test sample 1: b = 2.15, 95% CI: -71.30 – 56.46; test sample 2: b = 7.34, 95% CI: -66.34 – 68.19, meta-analysis: r = -.01, p = .91). Meta-analysis revealed a small to medium combined effect size for the total and direct effect (solid line, ** $p < .01$).



Supplementary Material Figure 2. Within-subjects mediation models shown separately for (A) pilot sample, (B) test sample 1 and (C) test sample 1. Combined results from the meta-analysis are shown in (D). Significant effects of social judgments (negative and neutral) on aggressive behavior (mean pressing time) are shown with solid lines (** p < .01, * p < .05). This relation was not mediated by frontal asymmetry (dotted lines, p > .05). The indirect effect was not significant (pilot sample: b = -11.93, 95% CI: -140.31 – 116.29; test sample 1: b = -34.27, 95% CI: -214.30 – 37.02; test sample 2: b = 11.17, 95% CI: -141.19 – 163.93, meta-analysis: r = -.01, p = .91). Meta-analysis revealed a small to medium combined effect size for the total and direct effect (solid line, ** p < .01).

APPENDICES

Nederlandse samenvatting (summary in Dutch)

Dankwoord (Acknowledgements)

Curriculum Vitae

Publicaties (publications)

Nederlandse samenvatting (summary in Dutch)

Elk kind reageert anders op zijn of haar omgeving. Sommige kinderen benaderen nieuwe situaties vol enthousiasme en plezier, terwijl andere kinderen voorzichtiger zijn en meer de kat uit de boom kijken. Of een kind in een bepaalde situatie op een gepaste manier reageert heeft te maken met het temperament en de sociale competenties van het kind. Wanneer iemand bijvoorbeeld hulp nodig heeft is het vaak wenselijk dat een kind naar diegene toe gaat om te helpen, maar sommige kinderen zijn te verlegen of bang om te helpen. Een ander voorbeeld: wanneer iemand een negatief oordeel geeft, is het vaak beter om rustig te blijven en terughoudend te zijn, maar sommige kinderen worden boos en gaan erop af om ruzie te maken. Eerder onderzoek heeft aangetoond dat een verschil in activiteit in de voorste delen van de rechter en linker helft van het brein, ook wel frontale asymmetrie (FA) genoemd, samenhangt met een verschil in het gedrag wat betreft toenadering zoeken of terughoudend zijn (zie voor een review Harmon-Jones, Gable, & Peterson, 2010). In dit proefschrift hebben we onderzocht of FA samenhangt met sociaal gedrag bij jonge kinderen, in de leeftijd van 4 tot 6 jaar oud. Tot dusver is er nog weinig bekend over de relatie tussen sociaal gedrag en FA in de vroege kindertijd. Wel weten we dat sociaal gedrag op jonge leeftijd een grote invloed heeft op sociale competenties later in het leven (Crick, 1996; Zentner & Shiner, 2015; Buss & Plomin, 2014; Dodge et al., 2003; Buckley, Winkel, & Leary, 2004). In dit proefschrift waren we met name geïnteresseerd in sociale reacties op buitensluiting of (negatieve) oordelen. Helpen kinderen anderen wanneer die worden buitengesloten door hen te includeren in het spel? En reageren kinderen agressiever nadat ze een negatief sociaal oordeel hebben gekregen dan na een positief sociaal oordeel? Om dit te kunnen meten hebben we bestaande taken speciaal aangepast voor kinderen van 4-6 jaar oud in ons onderzoek.

Tweelingenonderzoek

Leiden Consortium on Individual Development. De studies beschreven in dit proefschrift maken deel uit van een groter, longitudinaal onderzoek: het Consortium on Individual Development (CID), een consortium opgericht om te onderzoeken waarom het ene kind het beter doet dan het andere kind. In Leiden (L-CID) onderzoeken we de invloed van positief ouderschap op de sociale competenties en gedragscontrole van het kind (beschreven in het studieprotocol, zie Euser et al., 2016). In Leiden zijn er momenteel twee cohorten met elk 250 gezinnen met een tweeling van hetzelfde geslacht: een peuter/kleutercohort waarin de startleeftijd 3-4 jaar oud was en een basisschoolcohort waarin de startleeftijd 7-8 jaar oud was aan het begin van de studie. Door gebruik te maken van een experimenteel cohort-sequentieel design met tweelinggezinnen is het mogelijk om een gerandomiseerd onderzoek te doen (inclusief controlegroep) waarbij het longitudinale aspect is versneld. De metingen van de twee cohorten overlappen namelijk: de eerste twee meetmomenten van het basisschool cohort zijn hetzelfde als de laatste twee metingen van het peuter/kleuter cohort. Op deze manier verkrijgen we data van de gezinnen met tweelingen over de leeftijden van 3-14 jaar oud.

In L-CID hebben we gezinnen geïncludeerd met één- of twee-eiige tweelingen van hetzelfde geslacht. De gezinnen zijn geworven via gemeenteregisters in de randstad. Elk gezin is gevraagd om mee te doen aan een zesjarig onderzoek met jaarlijkse bezoeken. Deze bezoeken vonden thuis plaats of op de universiteit. Voor de studies beschreven in dit proefschrift is gebruik gemaakt van de data verzameld tijdens een pilot studie[4] en tijdens het tweede meetmoment van het peuter/kleutercohort waarin de kinderen de leeftijd van 4-6 jaar hadden. Dit meetmoment vond plaats op de universiteit en bestond onder andere uit een meting van hersenactiviteit door middel van een elektro-encefalogram (EEG).

Erfelijkheid. Doordat we gezinnen includeerden met één- of twee-eiige tweelingen konden we ook genetische en omgevingsinvloeden op bepaalde eigenschappen onderzoeken. Erfelijkheidsonderzoek is gebaseerd op het gegeven dat eeneiige tweelingen dezelfde genetische achtergrond hebben. Dit betekent dat eeneiige tweelingen een overlap van vrijwel 100% in hun genen hebben terwijl twee-eiige tweelingen gemiddeld maar een

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[4] een pilot studie is een studie om te testen of de taakjes geschikt zijn voor de doelgroep en om te kijken hoeveel tijd er nodig is voor een bezoek

overlap van 50% in hun genen hebben, net zoals gewone broers en zussen. De omgevingsinvloeden voor één- en twee-eiige tweelingen zijn gelijk, ze delen een bepaalde omgeving zoals het gezin of woongebied waarin ze opgroeien. Dit noemen we gedeelde omgeving. Elk kind heeft ook unieke ervaringen die verschillen binnen een tweeling, dit noemen we unieke omgeving. Wanneer een eeneiige tweeling meer overeenkomt (een hogere correlatie heeft) op een bepaalde eigenschap dan een twee-eiige tweeling dan wijst dat op een genetische invloed. Dit komt doordat deze grotere overeenkomst dan niet te verklaren is door de gedeelde of unieke omgeving (die is immers gelijk tussen één- en twee-eiige tweelingen). Wanneer de overeenkomst tussen eeneiige tweelingen veel groter is dan tussen twee-eiige tweelingen kunnen we zeggen dat een bepaalde eigenschap voor een deel erfelijk is. Met behulp van gedragsgenetica is het mogelijk om te kijken in hoeverre variatie in een eigenschap verklaard kan worden door genetische, gedeelde of unieke omgevingsfactoren. Daarnaast kan je onderzoeken of genetische of omgevingsinvloeden op twee eigenschappen overlappen, wat mogelijk een associatie tussen die twee eigenschappen kan verklaren.

Replicatie. Een ander voordeel van tweelingenonderzoek is dat het mogelijk is om de gevonden resultaten te repliceren door twee vergelijkbare groepen te creëren: elk kind van een tweelingpaar wordt dan willekeurig toegewezen aan een test- of replicatiegroep. We testen onze hypothese in de testgroep en kijken vervolgens of we dezelfde resultaten vinden in de replicatiegroep. Op deze manier optimaliseren we de kans op replicatie omdat de twee groepen hetzelfde zijn in achtergrondvariabelen zoals leeftijd, geslacht en woonomgeving. Het belang van replicatie in wetenschappelijk onderzoek is steeds duidelijker geworden. Dit komt mede door schandalen zoals de fraude-zaak van Diederik Stapel, maar ook andere zaken zorgden ervoor dat de maatschappij begon te twijfelen aan de betrouwbaarheid van wetenschappelijk onderzoek. In reactie op deze schandalen zijn er ideeën ontstaan om meer zekerheid te creëren binnen de wetenschap. Een van deze ideeën was het repliceren van de resultaten om de betrouwbaarheid van het onderzoek te verbeteren (Pashler & Wagenmakers, 2012). Als je namelijk hetzelfde resultaat vindt in twee groepen kan je er zekerder van zijn dat het resultaat solide is. Daarom hebben wij in dit proefschrift gebruik gemaakt van een replicatie-design.

Frontale asymmetrie

In dit proefschrift hebben we sociaal gedrag geassocieerd met frontale asymmetrie. Maar wat houdt frontale asymmetrie nu precies in? De activiteit van de frontale cortex, het voorste gedeelte van het brein, kan verschillend zijn tussen de linker en rechter hersenhelft. Dat betekent dat de linkerkant meer activatie kan vertonen dan de rechterkant, of andersom. Deze asymmetrie van de frontale hersenhelften wordt ook wel frontale asymmetrie genoemd, ofwel FA. FA kunnen we meten met behulp van een elektro-encefalogram (EEG), een methode om hersenactiviteit waar te nemen. Uit eerder onderzoek bij volwassenen en baby's is gevonden dat relatief meer activiteit van de linker frontale hersenen (linker FA) samenhangt met toenadering zoekend gedrag en dat relatief meer activatie van de rechter frontale hersenen (rechter FA) samenhangt met terughoudend gedrag (Davidson, Ekman, Saron, Senulis, & Friesen, 1990; Harmon-Jones et al., 2010; Harmon-Jones & Gable, 2018). Deze informatie hebben we gebruikt om te onderzoeken of er een relatie is tussen FA en bepaald sociaal gedrag of angstgevoelens bij jonge kinderen. De verwachting was dat angstgevoelens samen zouden hangen met terughoudend gedrag en dat angstgevoelens daarom geassocieerd zouden zijn met rechter FA. Daarnaast verwachtten we dat sociaal gedrag gericht op anderen, zoals iemand includeren bij buitensluiting of agressief reageren op negatieve oordelen, juist samen zou hangen met toenadering zoekend gedrag en dus geassocieerd zou zijn met linker FA. Deze hypotheses hebben we onderzocht in de drie studies beschreven in dit proefschrift.

Uitkomsten van het onderzoek

Angstgevoelens. In hoofdstuk 2 van dit proefschrift hebben we met behulp van gedragsgenetica onderzocht of er een overlap in genetische of omgevingsinvloeden is die een verband tussen angstgevoelens en FA zou kunnen verklaren. Met andere woorden: zijn er bepaalde genen die bepalen of een angstiger kind ook meer rechter FA laat zien? Angst geeft aan hoe nerveus of bezorgd iemand is met betrekking tot pijn, stress of dreigende situaties. Over het algemeen probeert men angstige situaties te vermijden, maar sommige kinderen zijn angstiger dan andere kinderen. Dit is onderdeel van het temperament van kinderen en kan invloed hebben op hun sociale gedrag. Eerder onderzoek liet zien dat angst samenhangt met rechter FA in volwassenen (Mathersul, Williams, Hopkinson, & Kemp, 2008) en kinderen (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001; Schmidt, 2008). Onderzoek bij kinderen laat echter inconsistente resultaten zien voor de relatie tussen angst en FA (Diaz & Bell, 2012; Howarth, Fettig, Curby, & Bell, 2016; LoBue, Coan, Thrasher, & DeLoache, 2011). In onze studie waren we ook geïnteresseerd in de gedragsgenetica rondom angstgevoelens. De resultaten lieten zien dat individuele verschillen in angstgevoelens en FA het best verklaard worden door een combinatie van genetische invloeden (ongeveer een kwart van de variantie tussen kinderen) en unieke omgevingsinvloeden (ongeveer driekwart van de variantie tussen kinderen). Tegen onze verwachting in vonden we hierin geen overlap: de genen en unieke omgevingsinvloeden die betrokken zijn bij angst zijn niet betrokken bij FA en vice versa. Dit houdt in dat onze data een verband tussen angstgevoelens en FA in de vroege kinderjaren niet ondersteunen.

Prosociaal gedrag in reactie op sociale buitensluiting. In hoofdstuk 3 hebben we de relatie tussen prosociaal gedrag en FA en de erfelijkheid van prosociaal gedrag onderzocht. Elke actie ten goede voor een ander persoon wordt gezien als prosociaal gedrag (Schroeder & Graziano, 2015). Hieronder valt bijvoorbeeld anderen helpen, samen delen, iemand gerust stellen of anderen betrekken bij een spel. Omdat prosociaal gedrag vaak begint met toenadering zoeken, was de verwachting dat het zou samenhangen met linker FA. Er zijn inderdaad een paar onderzoeken die prosociaal gedrag associëren met linker FA (Paulus, Kühn-Popp, Licata, Sodian, & Meinhardt, 2013; Huffmeijer, Alink, Tops, Bakermans-Kranenburg, & Van IJzendoorn, 2012). In ons onderzoek waren we met name geïnteresseerd in prosociaal gedrag in reactie op sociale buitensluiting. Eerdere onderzoeken bij volwassenen, pubers en oudere kinderen hebben aangetoond dat de meeste individuen compenseren voor sociale buitensluiting door de buitengesloten speler te includeren in het spel, gemeten met het Prosociale Cyberball-spel (Riem, Bakermans-Kranenburg, Huffmeijer, & Van IJzendoorn, 2013; Vrijhof et al., 2016; Van der Meulen, Van IJzendoorn, & Crone, 2016; Van der Meulen et al., 2017). Wij hebben dit spel aangepast voor jonge kinderen: het Prosociale Uilenspel. In het Prosociale Uilenspel spelen drie uiltjes met elkaar in de speeltuin, maar twee uiltjes sluiten een derde uiltje buiten. De participant (het kind dat meedoet aan ons onderzoek) heeft dan de mogelijkheid om de volgende beurt op het speeltoestel aan het buitengesloten uiltje te geven. De resultaten van ons onderzoek laten zien dat kinderen over het algemeen compenseren voor de sociale buitensluiting door het buitengesloten uiltje te kiezen in de eerste ronde van elk spel. Dit komt overeen met de resultaten van het Prosociale Cyberball-spel bij volwassenen en oudere kinderen (Riem et al., 2013; Vrijhof et al., 2016; Van der Meulen et al., 2016, 2017). In de tweede en derde ronde waren er individuele verschillen te zien. We hadden verwacht dat we deze verschillen tussen kinderen konden verklaren doordat er een relatie tussen prosociaal gedrag en FA zou zijn. Met andere woorden, kinderen met meer linker FA zouden eerder geneigd zijn om het buitengesloten uiltje te kiezen in de tweede en derde ronde van het spel. Onze resultaten lieten echter geen associatie zien tussen het Prosociale Uilenspel en FA. Wel vonden we dat prosociaal gedrag tijdens het Prosociale Uilenspel deels erfelijk is, eeneiige tweelingen kwamen dus meer overeen in hun prosociale gedrag dan twee-eiige tweelingen. Verder vonden we vrijwel dezelfde uitkomsten in de testgroep als in de replicatie-groep. Hiermee concluderen we dat het Prosociale Uilenspel een succesvolle aanpassing is van het Prosociale Cyberball-spel en gebruikt kan worden bij jongere kinderen.

Agressief gedrag in reactie op sociale oordelen. Naast de reactie op buitensluiting van een ander waren we ook geïnteresseerd in de reactie van de kinderen wanneer ze sociale oordelen van leeftijdsgenootjes ontvangen. Negatieve sociale oordelen van anderen kunnen resulteren in verdriet of boosheid, wat weer kan leiden tot agressief gedrag (Dodge et al., 2003; Buckley et al., 2004). Eerder onderzoek heeft laten zien dat volwassenen en oudere kinderen (7-10 jaar oud) agressiever reageren na een negatief sociaal oordeel dan na een positief sociaal oordeel van een ander, gemeten met de Social Network Aggression Task (SNAT; Achterberg, van Duijvenvoorde, Bakermans-Kranenburg, & Crone, 2016; Achterberg et al., 2017). Ander onderzoek heeft aangetoond dat agressief gedrag samenhangt met linker FA, omdat agressie gezien kan worden als toenaderend gedrag (Harmon-Jones & Sigelman, 2001; en zie ook de review van Harmon-Jones et al., 2010). In hoofdstuk 4 hebben wij gekeken hoe jonge kinderen reageren op sociale oordelen van leeftijdsgenootjes en we hebben dit gerelateerd aan FA. We hebben hiervoor de SNAT aangepast voor jonge kinderen (Social Network Aggression Task – Early Childhood ofwel SNAT-EC). Tijdens de SNAT-EC ontvangen de kinderen negatieve, positieve of neutrale sociale oordelen van fictieve/niet bestaande leeftijdgenootjes over hun knuffel (deze knuffel hebben ze eerst zelf uitgekozen). Na elk sociaal oordeel kreeg het kind de mogelijkheid om ballonnen van het leeftijdsgenootje kapot te maken: hoe langer het kind op een knop drukte hoe meer ballonnen er (virtueel) kapot gingen. Op deze manier bleven er minder ballonnen over voor het leeftijdsgenootje dat het oordeel had gegeven. Net zoals tijdens de SNAT voor oudere kinderen en volwassenen (Achterberg, Van Duijvenvoorde, Bakermans-Kranenburg, & Crone, 2016; Achterberg et al., 2017) hebben wij met de SNAT-EC laten zien dat kinderen agressiever reageren nadat ze een negatief sociaal oordeel hebben ontvangen in vergelijking met het ontvangen van een positief sociaal oordeel. Omdat FA een rol speelt in agressie hebben we onderzocht of kinderen na een negatief oordeel meer linker FA laten zien en daardoor ook meer ballonnen kapot maken. In tegenstelling tot onze verwachting was dit niet het geval; er werd geen verband met FA gevonden. In deze studie hebben we dezelfde onderzoeksvraag in drie groepen getoetst: een pilot-, test- en replicatiegroep. De data van die drie groepen hebben we gecombineerd door middel van een meta-analyse, waarmee je tot een betrouwbaardere conclusie dan wanneer je maar één groep analyseert. De meta-analyse over de drie groepen toonde aan dat de effectgrootte van sociale oordelen op agressie groot was en dat een negatief sociaal oordeel een agressievere reactie opwekt dan een positief sociaal oordeel. De SNAT is dus succesvol aangepast naar de SNAT-EC voor een jongere doelgroep.

Vervolgonderzoek

De studies in dit proefschrift hebben de rol van FA in sociaal gedrag onderzocht. Tegen onze verwachting in waren individuele verschillen tussen kinderen in angstgevoelens, prosociaal gedrag in reactie op sociale buitensluiting of agressief gedrag in reactie op sociale oordelen niet direct gerelateerd aan individuele verschillen in FA. Onze verwachtingen waren voornamelijk gebaseerd op onderzoek met volwassenen. Het is daarom belangrijk om in vervolgonderzoek de ontwikkeling van FA te onderzoeken, met name in de leeftijd van 4-6 jaar oud. Verder hebben we in onze studies steeds één meetmoment gebruikt. Wanneer je wilt kijken naar hoe bepaald (sociaal) gedrag zich ontwikkeld over de jaren is het interessant om naar meerdere meetmomenten te kijken. Dan is het namelijk ook mogelijk om te onderzoeken of FA op jongere leeftijd een voorspellende rol heeft in sociaal gedrag op oudere leeftijd. Dit is in de toekomst mogelijk in het longitudinale onderzoek van L-CID omdat de taken die we ontwikkeld hebben op meerdere leeftijden te gebruiken zijn. Helaas waren deze data nog niet beschikbaar voor het huidige proefschrift.

Conclusie

In dit proefschrift hebben we FA onderzocht in relatie tot angstgevoelens, prosociaal gedrag en agressief gedrag van kinderen van 4-6 jaar oud. Onze resultaten lieten geen verband zien tussen FA en sociaal gedrag. Wel hebben we aangetoond dat genetische invloeden betrokken zijn bij angstgevoelens, prosociaal gedrag en FA. Verder hebben we in dit proefschrift twee nieuwe taken ontwikkeld voor jonge kinderen: het Prosociale Uilenspel en de SNAT-EC. Beide taken lieten zien dan jonge kinderen vrijwel dezelfde reactie vertonen op sociale buitensluiting en negatieve sociale oordelen als volwassenen en oudere kinderen. Daarnaast hebben we binnen onze studies de resultaten kunnen repliceren. Hiermee kunnen we concluderen dat de taken een succesvolle aanpassing zijn van de volwassen versies. Het is dus mogelijk om de taken te gebruiken in longitudinale studies waarin we sociaal gedrag kunnen onderzoeken van de kindertijd tot aan volwassen leeftijd. De data die we in dit proefschrift hebben verzameld zal daarmee ook van toenemende waarde zijn in de komende jaren. Binnen de L-CID studie zal er namelijk steeds meer longitudinale data worden verzameld om sociaal gedrag te onderzoeken (zie ook het studieprotocol van Euser et al., 2016). De rol van FA in sociaal gedrag is op dit moment nog onduidelijk, maar nieuwe inzichten kunnen worden onthuld wanneer we naar de relatie tussen FA en sociaal gedrag kijken over een langere periode.

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Curriculum Vitae

Ilse van Wijk werd geboren op 9 november 1988 in Nieuwerkerk aan den IJssel. In 2007 behaalde zij haar VWO diploma aan het Veluws College in Apeldoorn. Aansluitend begon zij aan de bacheloropleiding Gezondheid en Leven aan de Vrije Universiteit Amsterdam en in 2010 aan de research master opleiding *Cognitive Neuropsychology* aan dezelfde universiteit. Tijdens haar research masteropleiding heeft Ilse een onderzoeksstage gedaan bij het *Baby Research Lab* aan de Radboud Universiteit in Nijmegen. Na haar afstuderen heeft Ilse gewerkt als onderzoeksassistent op de afdeling Biologische Psychologie van de Vrije Universiteit Amsterdam. Vervolgens is ze in 2014 gestart als promovenda op de afdeling Algemene en Gezinspedagogiek aan de Universiteit Leiden. Hier heeft ze asymmetrische frontale hersenactiviteit onderzocht in relatie tot angstgevoelens, prosociaal gedrag en agressief gedrag bij kinderen van 4-6 jaar oud. De resultaten van haar onderzoek zijn beschreven in dit proefschrift. De dataverzameling voor dit onderzoek vond plaats binnen het *Leiden Consortium on Individual Development* (L-CID). Momenteel is Ilse werkzaam als tutor bij de afdeling Psychologie en de afdeling Pedagogiek bij de Vrije Universiteit in Amsterdam.

Publicaties (Publications)

van Wijk, I. C., Huffmeijer, R., Bosdriesz, J. R., Bakermans-Kranenburg, M. J., Kolijn, L., van IJzendoorn, M. H., Vrijhof, C.I. & van den Bulk, B. G. (2019). Behavioral genetics of temperament and frontal asymmetry in early childhood. *Journal of experimental child psychology*, 179, 348-361.

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Ingediende manuscripts (manuscripts submitted for publication):

van Wijk, I. C., Euser, S., Huffmeijer, R., van den Bulk, B. G., Bakermans-Kranenburg, M. J., van IJzendoorn, M.H. Prosocial Owl Game: Assessing Compensation for Social Exclusion in Early Childhood.