RESEARCH PROTOCOL

The Neural Basis of Prosocial Development in Adolescence

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
(S)AE	(Serious) Adverse Event
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
WAIS	Wechsler Adult Intelligence Scale
WISC	Wechsler Intelligence Scale for Children
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale:

Despite the great value humans place on prosocial behavior (i.e. actions that benefit others, often at cost to oneself), science has long been puzzled by its existence (Simpson & Willer, 2008). Even though theories of kin selection (Hamilton, 1964) and reciprocal actions (Trivers, 1971) could partially explain the occurrence of prosocial behavior, they cannot explain why much prosocial behavior is directed at unrelated strangers (Simpson & Willer, 2008). Recent studies have suggested that adolescence may be an important period for the development of prosocial behavior (Güroğlu, van den Bos, & Crone, 2014). Adolescence is the period between ages 10-22 years during which most individuals shift from egocentric motivations to other-oriented behavior, and develop mature social goals. New insights have built upon an integrative account of how interactions between brain and behavior lead to long-term development of prosocial behavior (Crone & Dahl, 2012). In the present project, we aim to advance our understanding of the development of prosocial behavior by testing a new perspective on adolescent development, which argues that changes in emotional reactivity in response to prosocial actions pose opportunities for positive prosocial development. Specifically, we propose that the interaction between emotional reactivity and social cognitive control (i.e. the ability to take perspective of others) may result in adolescents' increased engagement in prosocial behavior.

Objective:

The aim of this proposal is threefold: (i) test a neuroscientific model of prosocial development by relating neuroscience discoveries to changes in several dimensions of prosocial development in a comprehensive study including children, adolescents and adults, (ii) test the moderating role of context (e.g. environmental support factors including parent and peer relations) and individual differences (e.g. in personality), and (iii) test for prosocial experience effects in an naturalistic test-retest study aimed at fostering prosocial development in adolescence.

Study design:

This study uses a comprehensive longitudinal design combining neural activity responses with behavioural assessments. Participants will perform computerized tasks related to prosocial behaviour and we will measure brain activation using functional Magnetic Resonance Imaging (fMRI) while they are performing the tasks. We will use structural MRI and Diffusion Tensor Imaging (DTI) to measure underlying brain anatomical processes. In addition, we will measure cognitive functioning on a battery of tasks outside of the scanner. We will also collect hormone measures from saliva and hair samples. All measurements are non-invasive.

Study population:

150 healthy volunteers, ages 9 – 18 years

150 mothers and 150 fathers of aforementioned healthy volunteers, 30+ year olds

Naturalistic test-retest study:

During 6 weeks, one group will once daily choose one of ten prosocial actions to carry out during the day, and will write a reflection about this early in the evening. The control group

will once daily choose one academic action from a list to carry out during the day, and will also write a reflection about this early in the evening.

Main study parameters/endpoints:

Age- and puberty related change in brain structure and function related to the construct of prosocial behaviour.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

There are no known risks associated with participating in the proposed measurements. MRI is a non-invasive technique involving no catheterizations or introduction of exogenous tracers. Numerous children and adults have undergone magnetic resonance studies without apparent harmful consequences. Some people become claustrophobic while inside the magnet and in these cases the study will be terminated immediately at the subject's request. The only absolute contraindications to MRI studies are the presence of intracranial or intraocular metal, or a pacemaker. Relative contraindications include pregnancy and claustrophobia. Subjects who may be pregnant, who may have metallic foreign bodies in the eyes or head, or who have cardiac pacemakers will be excluded because of potential contraindications of MRI in such subjects. Although there is no direct benefit to the participants from this proposed research, there are greater benefits to society from the potential knowledge gained from this study. This knowledge about typical development is critical to aid in the understanding of cases of atypical development, as seen in children with autism spectrum disorder, depression, schizophrenia, Attention Deficit Hyperactivity Disorder, Obsessive-Compulsive Disorder, Tourette’s syndrome, or traumatic brain injury. Secondly, the integrative knowledge on prosocial behaviour gained by this study is important not only for theory development, but also to eventually tailor societal programs (e.g., education, training) to the needs of adolescents.

1. INTRODUCTION AND RATIONALE

Adolescence as an important window for positive development

Adolescence is an important transition period between the dependency on parents and other caregivers of childhood, and the mature social goals and independence of adulthood. As such, adolescence represents a developmental time window typified by strong needs for exploration, forming new relationships, increasing intimacy, and rapid adjustment to changing social contexts (Blakemore & Mills, 2014; Steinberg, 2008). Adolescence starts with the onset of puberty, approximately at the age of 9-10-years in girls and 10-11-years in boys, although differences are observed between countries and cultures (Crone & Dahl, 2012). The onset of puberty is characterized by a rise in gonadal hormones, which are released through the hypothalamus-pituitary-gonadal axis and have large influence on bodily characteristics and brain development (Paus, 2013; Shirtcliff, Dahl, & Pollak, 2009)]. Pubertal development (also referred to as early to mid-adolescence) lasts until approximately age 15-16-years (with differences between cultures), but the prolonged period of mid- to late adolescence continues until individuals have achieved mature social and personal responsibilities. The period of adolescence has extended considerably in the last century, with longer reliance on parents and more possibilities for personal development and identity formation (Arnett, Žukauskienė, & Sugimura, 2014).

The dynamic characteristic of emotional reactivity is thought to peak in mid-adolescence (Ernst & Fudge, 2009; Galvan, 2010; Larson, Moneta, Richards, & Wilson, 2002). Traditionally, emotional reactivity has been linked to maladaptive adolescent behaviors such as alcohol and substance abuse, anxiety and depression (Giedd, Keshavan, & Paus, 2008).These behaviors are known to increase considerably in adolescence with peaks in risk-taking and social anxiety around age 16-17-years (Blöte, Kint, Miers, & Westenberg, 2009; Steinberg et al., 2008). This is also the time when most affective psychiatric disorders manifest themselves for the first time, such as anxiety disorder, depression, substance abuse and schizophrenia (Giedd, Keshavan, & Paus, 2008; Lee et al., 2014). Together, these findings have led to the hypothesis that adolescence may be a sensitive period for negative developmental consequences (Steinberg, 2011).

The existing models have often ignored how the aspects of normative development in emotional reactivity have adaptive functions, and in particular increase the potential for positive developmental outcomes. This proposal tests the hypothesis that the very same emotional reactivity that creates sensitivities for potential negative developmental trajectories (including risk for substance abuse, delinquency, social anxiety, or depression) may under other circumstances create opportunities for positive developmental trajectories – such as by fostering social sensitivity, cooperation, sharing and helping (Telzer, Fuligni, Lieberman, & Galván, 2014).

The development of prosocial behavior is of crucial importance for taking social responsibilities and developing mature social relationships and requires social-cognitive control (van IJzendoorn & Bakermans-Kranenburg, 2014). Whereas it has been well conceptualized that the basic social-cognitive building blocks for prosocial behavior, such as theory of mind, develop in early childhood, recent studies have supported the notion that

more complex social-cognitive behaviors emerge in adolescence (Dumontheil, Apperly, & Blakemore, 2010; Güroğlu, van den Bos, & Crone, 2009; van den Bos, Westenberg, van Dijk, & Crone, 2010). It is not yet known how and when these changes take place for prosocial development. In this proposal we will test the hypothesis that protracted development of social-cognitive functions create opportunities to aid rapid adaptation to different contexts (i.e., goal flexibility) (Crone & Dahl, 2012).

A cognitive neuroscience perspective on adolescent development

By the time children enter puberty, the size and shape of their brain is approximately the same as adults. Yet, numerous longitudinal structural neuroimaging studies have revealed that adolescent development involves widespread changes in the structure of the brain (Giedd et al., 2015; Mills & Tamnes, 2014; Tamnes et al., 2009). Longitudinal research examining changes in brain structure over time within individuals has shown that cortical white matter increases approximately linearly with age throughout childhood and adolescence (Paus, 2010). In contrast, cortical gray matter, which reflects neuronal density and the number of connections between neurons, follows an inverted-U shape over development, peaking at different ages depending on the region (Giedd et al., 2015). Therefore, gray matter loss is considered an index of the time-course of maturation of a brain region (Lee et al., 2014).

Within the cortex, gray matter reduction is most protracted for medial and lateral prefrontal cortex (PFC), and the junction between temporal cortex and parietal cortex (temporal-parietal junction: TPJ), in that cortical gray matter loss continues until the early 20s (Mills, Lalonde, Clasen, Giedd, & Blakemore, 2012; Gogtay et al., 2004). The development of subcortical brain regions, which are from an evolutionary perspective older parts of the brain, is also subject to both linear and nonlinear changes, such that some subcortical regions (such as the caudate and the putamen) linearly decrease in size, whereas other subcortical regions (such as the amygdala and the hippocampus) show an increase in size at the onset of puberty, and the growths stabilize in adolescence and adulthood (Østby et al., 2009; Tamnes et al., 2009). Both cortical and subcortical brain development is driven by both age and puberty specific changes (Blakemore, Burnett, & Dahl, 2010; Giedd et al., 2006). The relation between structural brain volume changes and changes in behavior, however, is currently not yet well understood. In addition, almost nothing is known about how environmental influences shape brain development in adolescence. Although it is likely that age-changes are partly related to experience dependent changes, the changes in brain structure in relation to individual experiences and behavioral outcomes have yet to be examined.

The relation between brain development and behavior has been studied using cross-sectional event-related functional magnetic resonance imaging (fMRI: a safe and non-invasive brain imaging technique) studies, including children, adolescents and adults. These studies have reported separate developmental pathways for emotional reactivity, with a focus on the subcortical ventral striatum, and social-cognitive control, with a focus on cortical brain regions including the PFC, the TPJ, and the superior temporal sulcus (STS). The ventral striatum is involved in many different types of affective and learning signals, but is well known for its role in processing a variety of basic rewards (Haber & Knutson, 2010). The

ventral striatum has anatomical and functional connections to the orbitofrontal cortex (Peper et al., 2012) and together this network of brain regions has been interpreted as having a crucial role in updating of reward values (Delgado, 2007; Haber & Knutson, 2010). A significant number of studies have found that, relative to children and adults, activity in ventral striatum is heightened during adolescence, suggesting more emotional reactivity in response to reward (Casey, Jones, & Sommerville, 2011; Galvan, 2010) This result has been replicated several times using a variety of gambling paradigms, such as passive gambling tasks (Galvan et al., 2006; van Leijenhorst et al., 2009) active gambling tasks (Ernst et al., 2005; van Leijenhorst et al., 2010), social risk taking tasks (Chein, Albert, O'Brien, Uckert, & Steinberg, 2011) and probabilistic learning tasks (Cohen et al., 2010). One potential mechanism for this heightened reactivity could be pubertal development and the associated rise in hormones, which correspond with activity in the ventral striatum (Op de Macks, 2011). Although most prior studies have relied on monetary rewards to elicit striatal reactivity, recent work shifted to other forms of rewards showing that the ventral striatum response appears to be highly sensitive to social factors, especially in adolescence (Chein et al., 2011). The proposed study will test whether emotional reactivity in adolescence in terms of ventral striatum activity can account for changes in the emotional valuing of prosocial activities. Even though this ventral striatum response to prosocial reward is reliably observed in adults (Lieberman & Eisenberger, 2009; Rilling & Sanfey, 2011), no study to date examined the developmental trajectory of ventral striatum activity in relation to valuing prosocial outcomes in adolescence.

There is considerable evidence from cognitive control studies that adolescents sometimes show reduced activity in lateral and medial PFC when performing a cognitive task, for example in tasks where the rules are explicit and the response options are constrained (Bunge & Wright, 2007). However, in other situations adolescents show elevated activity in PFC, for example when performing a divergent thinking task (Kleibeuker et al., 2013) or when performance is rewarded (Teslovich et al., 2014). Possibly, these differences reflect higher goal flexibility in adolescents relative to adults, with flexible recruitment of PFC, TPJ and STS, depending on whether situations require exploration and forming new (social) connections (Casey, 2015; Crone & Dahl, 2012). Heightened emotional reactivity in combination with flexible PFC, TPJ and STS recruitment may help adolescents to successfully navigate a complex social world which helps them to develop new social relations based on values such as cooperation and sharing. This flexible goal recruitment may be especially important when considering whether to engage in prosocial behavior such as sharing or helping others. This study will be the first to test the developmental trajectory of PFC,TPJ, and STS in relation to cooperation, sharing and helping in adolescence.

A cognitive neuroscience perspective on prosocial behavior

Prosocial behavior refers to behavior that benefits others which can be non-costly (e.g., helping) or costly (e.g., sharing). We will use three fMRI) paradigms to test the development of various dimensions of prosocial behavior. First, we will use a donating task to examine donating behavior in adolescence towards several targets (i.e., individuals the prosocial behavior is aimed at). Second, we will investigate whether movement synchronization (in

terms of finger tapping) will affect perceived self-other boundaries, and as a result prosocial behavior (operationalized as cooperation in a trust game). A third fMRI task tests for the development of two types of prosocial behavior, trust and reciprocity. For a more detailed description of the paradigms, see section 8.3 (*study procedures*).

To date, the relationship between brain structure and prosocial development has only been studied with a cross-sectional design (Thijssen et al., 2015) which limits the possibility to draw conclusion with respect to correlations versus predictors for change. These findings underline the crucial importance of longitudinal designs over cross-sectional designs and underline the value of neuroimaging as an additional tool for predicting change, over and above behavioral assessments.

Context and environmental factors: Is there a sensitive period for prosocial development?

In terms of early life experiences, it is well documented that attachment relationships with parents are important for shaping social behavior, given that parents provide proximity in times of need or presence of threats (Van IJzendoorn, & Bakermans-Kranenburg, 2014). There are indications that positive early life experiences, such as warm maternal affect, influence subsequent neural developmental trajectories (Tan et al., 2014). This study will test whether attachment relations with parents modulate neural activity related to prosocial development in adolescents. In addition, prior studies have reported that in adolescence attachment shifts partly to peers (Steinberg, 2008). This study will test whether peer relations modulate neural activity related to prosocial behavior in adolescents. These attachment and peer relations are expected to be important in explaining individual differences in developmental trajectories.

Furthermore, there are currently no studies that have examined the impact of prosocial experiences on brain development in adolescence and addressed the question if adolescence creates unique opportunities for certain types of social learning. If brain regions involved in emotional reactivity do peak in activity during that time, this leads to the hypothesis that adolescence is a (second) sensitive period in life where creating positive circumstances for prosocial development can have positive results in domains of social competence (Blakemore & Mills, 2013). This study will address the question whether the experience with prosocial activities in terms of service learning to the community can reveal if and when in adolescence there is a sensitive time for prosocial development (Layous, Nelson, Oberle, Schonert-Reichl, & Lyubomirsky, 2012). One study found that adolescents who have a greater sense of obligation to and identification with their family show larger ventral striatum responses to prosocial rewards (Telzer, Masten, Berkman, Lieberman, & Fuligni, 2010). Findings like these suggest that social experiences (with family members, friends or peers) in the adolescent period of heightened emotional reactivity may precipitate developmental trajectories that help the development of prosocial behavior, kindness and social responsibility. This study will test whether doing good for others in the community is associated with changes in prosocial behavior and concomitant neural changes (Bekkens, 2008; Layous et al., 2012).

An important determinant for the influence of prosocial experiences is the sensitivity to environmental influences, such as a reactive temperament, which may be particularly important at the onset of adolescence during which social-affective changes create a window that builds upon and amplifies social valuing. One possible mechanism for how the trajectories may develop positively, is that if prosocial experiences, such as cooperation, sharing and helping, are experienced as relatively more rewarding specifically during adolescence and more so for some adolescents (adolescents with a reactive temperament), then multiple encounters of these experiences may set the stage for a trajectory in which adolescents feel more committed to these prosocial goals also when developing into adulthood (Crone & Dahl, 2012). This hypothesis predicts that those adolescents who show highest emotional reactivity to prosocial rewards in early adolescence also show the largest benefit of prosocial experiences.

2. OBJECTIVES

Primary Objectives:

This study has three main aims. First of all, we aim to test a neuroscientific model of prosocial development by relating neuroscience discoveries to changes in several dimensions of prosocial development in a comprehensive study including children, adolescents and adults. This will help us to have a theoretically based understanding of how several forms and dimensions of prosocial behavior develop in relation to each other. Second, we want to test the moderating role of context (e.g. environmental support factors including parent and peer relations), and individual differences (e.g. in personality). Third, we want to test for prosocial experience effects in a naturalistic test-retest study aimed at fostering prosocial development in adolescence.

In order to address these three aims, we will acquire fMRI and structural MRI data, and behavioral responses of participants aged 9 – 18 (*N* =150) and their parents (mothers, *N* =150 and fathers *N* = 150). One year later, the participants will be followed up with a second scanning session (longitudinal design). After the second session participants will complete a naturalistic test-retest study to foster prosocial behavior or academic achievement. The naturalistic test-retest study will be followed by a final scanning session of the participants, which will allow us to examine effects of naturalistic test-retest study prosocial experiences on prosocial behavior and the brain. These findings will be complemented with hormonal variables and performance outside of the scanner on a battery of tests.

In addition, a pilot study will be performed including 20-25 year-old adults (*N* = 30).

3. STUDY DESIGN

This study will make use of a cohort-sequential longitudinal design to investigate prosocial development and its neural basis in adolescence. The advantage of this design is that it efficiently captures developmental trajectories in a short period of time (i.e., within 1.5 years, in which the second and third scan (T2, T3) take place one year after the first (T1), and T3 takes place 2 months after T2; see Table 1). A particular strength of this design is the unique opportunity to test prosocial interactions across various domains, individuals, age groups, and time.

Before the start of the longitudinal study, the experimental tasks and procedure for the adolescents will be validated in 30 adults (15 female, 15 male) between ages 20 – 25.

Summary

- Participants are invited to a test session three times
- During lab sessions, participants will perform cognitive and behavioral tasks
- During the three lab sessions, participants will perform several tasks in the scanner. Three indices of functional brain development will be obtained in all individuals, which are expected to measure several dimensions of prosocial behavior (e.g. donating, trusting, and cooperating).
- Neural activity in the proposed tasks will be complemented with self-report indices to examine the unique predictive value of brain activity, and the commonality between brain activity and self-report ratings, within and across individuals.
- We will assess structural brain data to test for differences and changes in brain architecture. High-resolution structural brain imaging will allow us to examine cortical gray and white matter volume, and diffusion tensor imaging (DTI) will allow us to examine the white matter pathways in the brain. Resting-state fMRI will be assessed to allow for the mapping of the functional connectivity of the brain, reflected in the correlation between temporal activation patterns in different brain areas independent of a specific task.
- To assess puberty stage and hormone levels, we will make use of self-report data (see Appendix F), and saliva and hair samples. Saliva will be collected by passive drool and will be analyzed for levels of testosterone, dehydroepiadrosteronsulfaat (DHEAS), oxytocin, cortisol, and estradiol. To account for daily hormone fluctuations we will collect salvia samples on multiple time points on the same day, and the longitudinal design will allow us to test within-subject changes in hormone levels that account for baseline differences between individuals. Next to the current hormone levels determined in saliva, which thus fluctuate during the day, hair samples will be used to obtain information on long-term hormone secretion. Hormone concentrations in hair provide a more stable indicator of hormone secretion over a prolonged period of time, and can be related to the effects of our naturalistic test-retest study (T2-T3). In this way, both participants' state-dependent hormone fluctuations and (trait-like) stable hormonal profiles can be captured.

4. STUDY POPULATION

4.1 Population (base)

We aim at including a total of 450 participants: 150 adolescents between the ages 9 and 18 years. Both genders will be represented equally within each age group. In addition, we aim to include the (150) mothers and (150) fathers of the adolescent participants.

Participants will be selected in ongoing collaborations with local schools (Rijnlands Lyceum Wassenaar; Rijnlands Lyceum Oegstgeest; Da Vinci College Leiden, Stedelijk Gymnasium Schiedam, Fioretti College Lisse).

Potential participants will be prescreened on the telephone for contraindications for (f)MRI, including metal implants, heart arrhythmia, claustrophobia, and possible pregnancy. In private telephone conversations, adolescent females will be told that if they are interested in participating in the study, they will be prescreened for pregnancy and other MRI

contraindications. They will be advised not to participate if there is a chance they may be pregnant. To prevent scanning girls who could be pregnant without knowing it or reporting it, we will schedule the scanning session within 5-10 days following the start of menstruation. This has the additional advantage that hormone levels are relatively low which excludes noise related to changes within the cycle. Participants will be additionally prescreened for head trauma, premature birth, learning disabilities, and history of neurological or psychiatric illness and/or use of psychotropic medications.

Following standard procedures, all measures will be assessed in private settings and with informed consent from parents. The infrastructure at the Brain & Development Research Center of Leiden University will enable us to test the proposed number of participants in the time frame as indicated.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Fluent Dutch speaker
- For adolescents: starting age between 9- 17 years at the first session (T1)
- For adults (pilot): starting age between 20 – 25 years

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Participants with a history of neurological or psychiatric disorder/disease or current use of psychotropic medications.
- Contraindications for MRI, including metal implants, heart arrhythmia, and claustrophobia.
- Females who are pregnant.
- Participants will additionally be pre-screened with a checklist for head trauma, premature birth, and learning disabilities.

4.4 Sample size calculation

We propose to collect data from 150 adolescents and (only at T1) their (150) mothers and (150) fathers. The adolescents will be 75 healthy boys and 75 healthy girls, and will be divided over three cohorts: Cohort 1 (9-11 years old at T1), Cohort 2 (12-14 years old at T1) and Cohort 3 (15-17 years old at T1). The adolescent participants will be followed up after one year (T2). After T2, adolescent participants will take part in a naturalistic test-retest study that will take 6 weeks. The naturalistic test-retest study will be followed by another test session at T3. Data from parents will only be collected once, at T1. See Table 1 for a representation of the adolescent age groups at each time point.

In a prior longitudinal fMRI study from the Brain and Development laboratory, 299 children were tested in year 1 and there was a follow up of 254 participants 2-3 years later. The reasons for drop out were mostly braces (n=35), and a small number of participants who moved and were therefore no longer traceable (n=7), or who preferred not to participate anymore (n=3). Therefore, we predict attrition of <20% for the longitudinal follow-up, which should result in enough data points in all test sessions.

Table 1. Anticipated sample sizes for each age group at the three time points, T1 – T3. C1 = Cohort 1, C2= Cohort 2, C3= Cohort 3.

	Adolescents						**Parents**	**A + P Combined**
Age group	9 - 11	10-12	12-14	13-15	15-17	16-18	30+	**Total N**
T1	C1: 50		C2: 50		C3: 50		2x150	**450**
T2		C1: 50		C2: 50		C3: 50		**150**
T3		C1: 50		C2: 50		C3: 50		**150**
Total N per age	50	50	50	50	50	50	300	600
Total N attrition-corrected	50	40	50	40	50	40	225	495
Total Data-points per age	50	100	50	100	50	100	300	750
Total Data-points attrition-corrected	50	80	50	80	50	80	225	615

Note: Participants will have approximately the same age at T2 and T3 because these time-points will be 6 weeks apart in time.

Power analyses conducted on actual event-related fMRI data suggest that the benefit of adding additional subjects plateaus after 25 subjects (Murphy & Garavan, 2004). The results of this analysis correspond well with a prior study on fMRI sample size, which suggested that up to 22 subjects are needed to achieve 80% power at an alpha of .05 (Lombardo et al., 2016). Based on these analyses, it is expected that in order to examine age- related change and gender differences in brain structure and function related to the construct of prosocial behaviour 20-25 subjects per age group and gender (i.e., 40 – 50 participants in total per age group) will be sufficient for reliable within-group average fMRI data at $p < .05$, cluster-corrected. As we expect a maximum attrition of 20% in our study for T2 and T3 (and thus a minimum of 40 participants per age group) we assume we will have enough power for age-related analyses (> 80%) and to examine gender effects (about 80%).

To test for initial age group differences, we will use two techniques for data analysis. The first is a regression analysis, which is based on the hypothesis that age will be a significant predictor of task-related brain activation. For this purpose, it is important to have enough participants of each age in the range. The second technique is an analysis of variance, in which we can include within-subjects variables, such as condition. For the latter technique, we will subdivide the children in separate age groups and compare them statistically. The statistical power for analysis of variance has been estimated by others (e.g. Murphy &

Garavan, 2004), and given that regression techniques have more statistical power, we expect that the same number of participants will be sufficient for both techniques.

Parents will only participate at the first time-point. It is expected that not all parents will be able to participate in the study, for example because of contra-indications for MRI. Hence, we hope that around 225 parents will be able to participate at t1, attrition-corrected.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

In this study we will make use of a behavioural naturalistic test-retest study that aims to foster prosocial behaviour in adolescents. For this naturalistic test-retest study, participants will be assigned to one of two conditions: half of participants will be assigned to a *prosocial* condition (i.e., performing prosocial actions such as giving a peer a compliment), and half of participants will be assigned to an *academic* condition (i.e., performing academic actions such as doing a homework assignment for which you can get extra credits). During six weeks, participants will be asked on each morning to perform an action from a list of either prosocial or academic actions, depending on the condition that they are assigned to. In the evenings, participants will be asked to report on whether they performed one of the actions on the list, which action they performed, and how they experienced this. Note that we only ask participants to report on the actions that happen in their daily life.

5.2 Use of co-intervention (if applicable)

N/A

5.3 Escape medication (if applicable)

N/A

6. INVESTIGATIONAL PRODUCT

For all chapters under 6: N/A

7. NON-INVESTIGATIONAL PRODUCT

For all chapters under 7: N/A

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameters

Developmental processes assessed with structural and functional brain imaging measures, behavioural and hormonal measures, and self-report questionnaires

8.1.2 Secondary study parameters

Assessments of *personality and social relations* (parent-, sibling-, and peer-relationships) using resting state fMRI and self-report instruments

8.2 Randomisation, blinding and treatment allocation

During all phases of the study, participants will be told that we are interested in how they socially interact with others (see information letters for participants). No emphasis will be placed on the prosocial nature of the measures and research, as this might bias participants' behaviour. In addition, we will assess the extent to which participants tend to give socially desirable answers.

For the naturalistic test-retest study, we will assign participants to one of two groups: 1) the group that receives the prosocial naturalistic test-retest study, 2) a control group that receives a similar program that is focused on academic choices. These groups will be matched on age, gender, and social value orientation. Participants will not be informed about the condition/group they are in. Participants will be fully debriefed about the naturalistic test-retest study after T3.

8.3 Study procedures

8.3.1 Developmental processes

Structural MRI

Two indices of structural brain development will be obtained. High resolution T1-weighted structural brain imaging will allow us to examine cortical gray matter and subcortical volumes (Shaw et al., 2008), and Diffusion Tensor Imaging (DTI) will allow us to examine the white matter pathways in the brain. Importantly, greater coherence in these pathways is associated with better performance on tasks that require an interaction between regions connected by these tracks (Peper et al., 2012; Olesen, Nagy, Westerberg, & Klingberg, 2003) and therefore will be particularly important in examining the connectivity between regions during functional MRI tasks.

Task-based fMRI paradigms

We will use three paradigms to test the behavioral and neural development of prosocial behavior.

Giving task

First, we will use a donating task to examine giving behavior in adolescence towards several targets (i.e., individuals the prosocial behavior is aimed at): the participants' mother/father, a friend, and a stranger. In this task, participants will divide a certain amount of resources between themselves and the target individual. The task will consist of two conditions: 1) an alone condition, in which participants are allowed to make their decisions anonymously, and 2) a public condition, in which participants are being watched by peers while the participants decide how to divide the resources. This design was piloted by means of a behavioral study, which showed that adolescents gave more coins to targets that they had a closer relationship with (e.g. they donated more coins to friends than to a stranger). In terms of brain activation, based on previous studies we expect that donations to others with whom participants have close relationships will be accompanied by ventral striatum (VS) activation, whereas donations to strangers will be accompanied by activation in areas that are important for self-control (VLPFC, ACC) and mentalizing (TPJ, DMPFC) (Telzer, Ichien, & Qu, 2015). Regarding the alone versus public conditions, we expect that VS activation will be higher in the public condition than in the alone condition (Chein et al., 2011). This study will test how the visibility of the participants' choice (i.e., whether it is made in the alone or public condition) will interact with choices for different targets (i.e., stranger, mother/father, friend). Feasibility of the fMRI design will be assessed in a pilot study with 30 adult participants, aged 20 – 25 (15 males, 15 females).

Trust and Cooperation Game

A paradigm that allows for the study of intentions in social interactions is the two player trust game (TG) paradigm, which taps into both the trust and reciprocity elements of social interactions (Berg, Dickhaut & McCabe, 1995). The structure of the TG is as follows: At the beginning of the TG, the first player (trustor) is endowed with a sum of money (e.g., €10). The first player is then presented with two options —he can either directly divide that sum according to a predetermined scheme (e.g., €5 for each player) or he can trust the second player (trustee) and give him the choice to divide the money. The latter option, to trust the other player, potentially leads to a higher pay off for both players because the sum of money is multiplied (typically tripled, in this case to €30). However, if trusted, the second player has two options: (1) reciprocate the trust given by the first player (e.g., €15 for each player) or (2) defect and maximize personal gains (e.g., €30 for the second player and nothing for the first player). As a result, trusting involves a component of risk because the trustee may attain higher personal benefit when not reciprocating (Rousseau et al., 1998).

If players in the TG were both completely driven by self-interest they would always defect as trustees and therefore never trust as trustors. However, it was demonstrated that even for single anonymous transactions individuals often trust and reciprocate (Berg, Dickhaut & McCabe, 1995). It has therefore been suggested that our motives to trust and reciprocate are not only guided by goals to maximize

personal outcomes but also by “other-regarding preferences” (Falk & Fischbacher, 2006; Fehr & Camerer, 2007; Van Lange, 1999; Fehr & Gintis, 2007). According to these studies the decision to reciprocate is dependent on evaluating consequences for both self and others.

Developmental studies using the TG have demonstrated an increase in both trust and reciprocity with increasing age, stabilizing around 22 years (Sutter & Kocher, 2007), following the development of social perspective taking, or the ability of integrating perspectives of self and other, which develops until late adolescence (Fett et al., 2014a).

Several frontal brain regions are involved in trust, reciprocity, and defecting trust, such as the anterior medial prefrontal cortex, dorsolateral prefrontal cortex, and anterior cingulate cortex, together with brain areas involved in perspective taking (TPJ) and social decision-making (insula, ventral striatum) (van den Bos et al. 2009). Developmental changes across adolescence in these brain regions might underlie the observed developmental differences in perspective taking and understanding of intentionality (Fett et al., 2014b).

In the current study, we aim to investigate whether participants’ choices to trust and reciprocate trust of another unknown and sex- and age-matched player can be manipulated by experimentally shifting the perceived boundaries between self and other through movement synchronization. Thus, participants will play the TG both in the role of the trustor and the trustee. Previous studies suggested interpersonal motor synchrony, by an increased sense of similarity between self and other, evoked compassion and affiliation, strengthened social attachment, and promoted prosocial and altruistic behaviors (Hove and Risen, 2009; Wiltermuth and Heath, 2009; Valdesolo, Ouyang, & Desteno, 2010; Valdesolo & Desteno, 2011; Cirelli, Einarson, & Trainor, 2014; Rabinowitch & Knafo-Noam, 2015). A recent behavioral study in 4 year old children showed that cooperation (as measured by the speed of a joint task completion) between a child and another unknown peer improved after the two children experienced synchronous motion as compared to asynchronous motion (i.e. they were swinging next to each other in synchrony or asynchrony), or no motion at all (Rabinowitch & Metzhoff, 2017). Research on the underlying neural mechanisms of interpersonal synchrony is scarce, but it was suggested that brain regions processing rewards were more strongly engaged during the experience of interpersonal synchrony, and that activation in reward processing brain areas was predictive for subsequent prosocial behavior (Kokal, Engel, Kirschner, & Keyser, 2011).

In the current study, participants will perform a finger tapping task (based upon the experimental design of (Rabinowitch & Knafo-Noam, 2015) in the MRI scanner. Pre-recorded video clips of an age- and sex-matched unfamiliar peer who is also performing the tapping task will be shown on one half of the screen (face and tapping hand are clearly visible). Tapping occurs together with a bouncing ball that is displayed on the other half of the participant’s screen. The ball jumps up and down in an isochronous manner (constant interval between each bounce) and the participant

is asked to tap each time the ball reaches the floor. To make this moment more distinguishable, the floor also turns red at the time of each expected tap. Each of three conditions (a. synchronous tapping with the other child, b. asynchronous with the other child, c. tapping while the other child is not tapping at all) consists of 2 tapping blocks of 1.5 minutes long rhythmic interactions with a brief interval between them for resting the hands. After each tapping condition, participants perform several trials of the TG and are told their playing partner is the same person as they performed the tapping task with. A picture of that person is shown on the screen during all trials of the TG. Unbeknownst to the participants, the decisions of the other person/player are not the decisions of a real participant, but are preprogrammed. After the scan/each condition, participants rate the picture of their playing partner on how much they liked the other person, how similar they perceive the other to be as themselves, and how close they feel towards the other.

Several hypotheses can be formulated. Based on prior studies in adults that demonstrated that cooperation is associated with activity in the ventral striatum (Rilling et al., 2004), and based on our own findings that between ages 10 and 16 years, there was a progressively quicker adjustment towards prosocial interaction with cooperating others (van den Bos et al., 2012), we hypothesize that ventral striatum response to reciprocate (in the baseline/control condition without any tapping interaction) increases between ages 10 and 16 years and peaks or plateaus in mid-adolescence. Second, we expect that ventral striatum activity during cooperation is further modulated by previous synchronous interaction with the unfamiliar peer, such that ventral striatum activity increases with more synchrony. A further hypothesis is that the increased reward value of reciprocity/being trusted is moderated by an increased feeling of similarity and closeness towards the other player. We also expect that after synchronous interaction, participants will more often choose to trust the other player and to reciprocate.

Vicarious Reward Task

The third task that will be used in the MRI scanner is a vicarious reward task (Braams, Peters, Peper, Güroğlu, & Crone, 2014). In this task, participants can choose heads or tails and win (or lose) money when the computer selects the chosen (or not chosen) side of the coin. As such, the chance of winning or losing will be 50% for each trial. Before the start of the task, participants will be told that they will play this task with various interaction partners (i.e., a friend, their mother/father, or a stranger). In each trial, they will be presented with feedback on how much they won or lost for themselves, a friend, their mother, their father, or a stranger. The amount of coins that they can win or lose will be varied over trials to make the task more engaging. As a baseline, there will also be trials in which participants will not gain or lose coins for themselves or the other (+0 coins or -0 coins trials). Participants' brain activation during the feedback can be compared with brain activation during the giving task to gain a better understanding of how voluntary (represented in the giving task) versus unvoluntary giving (represented in the vicarious reward task) differ from each

other and how they are related to adolescents' prosocial behavior. Previous studies using this task have shown activation (specifically, a mid- to late- adolescent peak in activation) in the ventral striatum as a result of winning for self or close others, and suggest that the medial prefrontal cortex is activated during winning for people that adolescents do not feel very close to (Braams et al., 2014; Braams & Crone, 2017).

All fMRI paradigms will be followed up outside the scanner with exit questionnaires in which participants get to elucidate the choices that they made during the tasks and how much they liked the various outcomes.

Behavioral tasks outside of the scanner

In order to obtain a comprehensive understanding of prosocial behavior, participants will complete a battery of tasks designed to measure prosocial behavior, including:

- a prosocial Cyberball Game assessing helping behavior (van der Meulen, van IJzendoorn, & Crone, 2016)
- a trust game assissing reciprocity (van den Bos et al., 2010)
- a dictator game targeted at charities (Eckel & Grossman, 1996)
- an extension of the donating task described under the task-based MRI paradigms which includes a non-costly donation condition (i.e., a condition is added in which the number of donated coins does not incur a loss for the participants)
- a naturalistic helping-task (e.g. in which the experimenter drops certain objects and observes whether the participant helps to retrieve the objects from the floor (Greitemeyer & Osswald, 2010).
- Risk-taking will be assessed using the Balloon Analogue Risk Task (Lejuez et al., 2002; Peper, Koolschijn, & Crone, 2015).
- A Go-NoGo task which measures the ability to inhibit prepotent responses (Casey et al., 1997).
- An ecologically appropriate social dilemma game: Iterated cleaning dilemma (based on the snowdrift dilemma, see: Maynard Smith 1982, Sugden 1986).

The collection of saliva by passive drool (Peper, Koolschijn & Crone, 2013) on different time points during the day will give information on the diurnal rhythm of the hormones of interest. Levels of testosterone, DHEAS, cortisol, oxytocin and estradiol will be assessed. To obtain information on long-term hormone secretion, hair samples can be used. Hormone concentrations in hair are more stable than in saliva and provide insight in the integrated hormone secretion over prolonged periods of time (Russell, Koren, Rieder, & Van Uum, 2012; Gao, 2013; Groeneveld et al., 2013). We will collect samples of approximately 50-100 hairs from the back of the head. The hair sample can only be collected when the child's hair is at least 3 cm long.
Hair and saliva samples will be stored until hormone assessments have been performed. Afterwards, all biomaterial will be disposed.

Self-report measures

- At all sessions, participants will complete a background questionnaire, measuring among other things date of birth, ethnicity, social economic status, social media use, and educational level and functioning. This questionnaire will also contain questions about participants' financial situation and responsibilities (i.e., how do participants regard money and their own financial situation? Do adolescents have financial responsibilities to pay for example their own clothing or mobile phone?), as these could influence how participants distribute resources or money in behavioral tasks and fMRI paradigms.
- Moreover, participants will complete a questionnaire on religion: the Religious Background and Behavior self-report questionnaire (Document F1.1, Connors, Tonigan, & Miller, 1996), which measures religious thought and behavior. This questionnaire distinguishes well between several worldviews e.g. religious, spiritual, agnostic, atheistic, and between current and past religiosity.
- In addition, participants will complete a battery of tasks designed to assess intelligence, such as sub-tests of the WISC/WAIS IV (Wechsler et al., 2003) and the Quick Word Test (Borgatta & Corsini) to assess language ability.
- Social desirability will be assessed with the Five-Item Measure of Socially Desirable Response Set (Hays, Hayashi, & Stewart, 1989).
- Furthermore, the Prosocial Tendencies Measure Revised (PTM-R, Document F1.2) will be used to assess participants' inclination to show six types of prosocial behaviors (Carlo, Hausmann, Christiansen, & Randall, 2003).
- Morality will be assessed with the measure of Prosocial Moral Reasoning (PROM-R, Document F1.3), in which participants are asked to explain other people's motivations to (not) display prosocial behavior (Eisenberg, Carlo, Murphy, & van Court, 1995).
- In addition, during the naturalistic test-retest study, participants will report and reflect on their prosocial experiences (or academic experiences if they are in the control condition) during the day by means of daily diaries.

Please note that questionnaires will be divided over several sessions in order to reduce pressure on the participant and to ensure that participants are able to fill out the questionnaires in a concentrated manner without feeling strained.

8.3.1 Personality & Social relations

Resting state fMRI

In addition to task-specific functional measures, resting-state fMRI will be assessed, which in adults and children has proven to be an informative method for mapping functional connectivity in the brain independent of a specific task (Fair et al., 2007). This connectivity is reflected in correlations between temporal activation patterns in different brain areas during rest, which gives an indication of connectivity strength and variability (Allen et al., 2014). A 'network' approach has the advantage of examining the spatial and temporal relationships among elements of the network. An important advantage of mapping resting-state activity is that it is highly reproducible and not

task-dependent (Damoiseaux et al., 2006); therefore, it can be of great value in understanding general developmental changes in brain functioning. The resting-state scans will be included in the longitudinal design in order to examine developmental differences in brain connectivity, and will be related to functional MRI results in the three tasks.

Personality questionnaires

- the Social Value Orientation Slider will be used to measure participants' preferences for the distribution of resources (Murphy, Ackermann, & Handgraaf, 2011).
- Empathy will be measured with the Empathy Questionnaire for Children and Adolescents (EmQue-CA, Document F1.12) (Overgaauw, Rieffe, Broekhoef, Crone, & Güroğlu, 2017)
- Also, the Feeling and Thinking questionnaire (Document F1.17, Garton & Gringart, 2005) will be used to assess both the cognitive and affective components of empathy
- the Brief Shame and Guilt Questionnaire (Document F1.18, Novin & Rieffe, 2015) will assess shame and guilt proneness
- to measure sensitivity to social rewards, we will use the Social Reward Questionnaire, (Document F1.11, Foulkes, Viding, McCrory, & Neumann, 2014)
- perspective taking will be measured with using the perspective taking subscale of the Interpersonal Reactivity Index (Document F1.13, IRI-PT; Davis, 1983)
- The Big Five Inventory (BFI, Document F1.15, John & Srivastava, 1999), a self-report, will be assessed to measure an individual on the Big Five dimensions of personality. These dimensions are: "Extraversion vs. introversion Gregariousness (sociable)", "Agreeableness vs. antagonism Trust (forgiving)", "Conscientiousness vs. lack of direction Competence (efficient)", "Neuroticism vs. emotional stability Anxiety (tense)", and "Openness vs. closedness to experience Ideas (curious)". The BFI consists of 30 items measured on a 7-point Likert scale (7 = strongly agree, 1 = strongly disagree).
- The Adolescent Risk-Taking Questionnaire (ATQ, Document F1.27) will be used to assess risk perceptions and potential for risky behavior (Gullone, Moore, Moss, & Boyd, 2000)
- The Adolescent Temperament Measure assesses temperament and self-regulation (Document F1.16, Simonds, 2006; Kapaldi & Rothbart, 1992).
- Finally, adolescents will fill out a questionnaire about goal-setting that is yet to be developed.

Social competence and social relations questionnaires

- Peer relations will be assessed with the self-report Friendship Quality Scale (FQS, Document F1.6) (Braams et al., 2014),
- with the Resistance to Peer Influence Scale (Document F1.7, Steinberg & Monahan, 2007),

- the Inventory of Parent and Peer Attachment Revised (Document F1.8, Gullone & Robinson, 2005),
- and the Olweus Bully/Victim Questionnaire (Document F1.9, Olweus, 1989).
- also, the Inclusion of Other in Self scale (Document F1.5, Aaron, Aaron & Smollan, 1992), for an indication of relationship closeness will be used
- the Experiences in Close Relationships – Structures Questionnaire (ECR-RS, Document F1.10), Fraley et al., 2011) will be used to measure attachment patterns in a variety of close relationships (i.e. mother, father, siblings, best friends).
- In addition, questions will be asked about participants' number of friends, time spent with friends, peer networks and popularity. Also, sociometric data will be obtained.
- different facets of the parent-child relationship will be assessed using the Cohesion subscale of the Family Adaptation and Cohesion Evaluation Scales inventory (FACE IV, Document F1.4, Olson, 2011),
- Another 10 item questionaire assesses familiy conflict (Ruiz et al. 1998, Telzer et al., 2014)
- an 8 item scale on family identity (Tyler & Degoey, 1995) will be used
- finally, we used a parenting style questionnaire, based mainly on the Leuven Adolescent Perceived Parenting Scale (LAPPS, Delhaye et al., 2012; the instrument used here combines scales of several other parenting style instruments, Document F1.28)

Pubertal assessment

Participants will be assigned to pubertal maturation groups based on a compound score of different puberty assessment indices. Participants will be asked to complete a self-report measure of pubertal maturation.

- The self-report scale is the Pubertal Development Scale (PDS, Document F1.19, Petersen et al., 1988). The PDS consists of five questions about physical development, scores from 1 (no) to 4 (development seems complete). In prior research, reliability of the PDS was high (.77 for boys, .81 for girls). Prior research has demonstrated that the self-report data provide similar or even better indices of pubertal maturation than when the assessment is done by a nurse practitioner in the form of a physical examination, possibly because self-assessments are based on more continuous judgements than a one-visit decision (Shirtcliff, Dahl & Pollak, 2009). One concern when assessing pubertal maturation based on self-report is that early maturing adolescents are inclined to underestimate pubertal maturation and late maturing adolescents are inclined to overestimate pubertal maturation (Shirtcliff, Dahl & Pollak, 2009).

8.3.2 Parents

The above described procedure for adolescent participants is similar for their participating parents, with some exceptions:

- In the donation fMRI task, the targets will be the participant's child, their friend, and a stranger.
- The MyChild questionnaire will be used to measure children's empathic- and prosocial behavior, as well as feelings of guilt and internalized conduct (Document F1.20, Kochanska, DeVet, Goldman, Murray, & Putnam; 1994)
- The Parenting Scale (Document F1.21, Prinzie, Onghena, & Hellinckx, 2007) will be used to assess parenting styles
- The Adult Temperament Questionnaire (Document F1.22, Evans & Rothbart, 2007) will be used to measure parents' temperament and behavioral inclinations.
- The Investment Model Scale (Document F1.23, Rusbult, Martz, & Agnew, 1998) will be used to assess parents' perceived relationship quality with their partner.
- The Strenghts and Difficulties Questionnaire (SDQ, Document F1.24) and Child Behavior Checklist (CBCL, Document F1.25) will be used to measure the child's problem behavior (Achenbach et al., 2008, 2011).
- The Social Responsiveness Scale (Document F1.26, Constantino et al., 2005) will be used to measure children's social responsiveness and autistic traits.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal: N/A

8.5 Replacement of individual subjects after withdrawal

N/A

8.6 Follow-up of subjects withdrawn from treatment

N/A

8.7 Premature termination of the study

The study will be terminated prematurely only in case of technical problems with the MRI equipment, such as the MRI scanner itself or the computer that presents the stimuli. In case of an error that is not directly solvable, the subject session will be ended prematurely.

SAFETY REPORTING

8.8 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take

care that all subjects are kept informed.

8.9 AEs, SAEs and SUSARs

8.9.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental naturalistic test-retest study]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.9.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical naturalistic test-retest study but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.
The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

8.9.3 Suspected unexpected serious adverse reactions (SUSARs): N/A

8.10 Annual safety report: N/A

8.11 [Data Safety Monitoring Board (DSMB) / Safety Committee] : N/A

8.12 Unexpected findings

Under responsibility of dr. M. Kruit (LUMC, Dept. of Radiology) anatomical scans will be examined by a physician for unexpected findings. In the 'proefpersoon informatiebrief', the research participants will be informed that the scans are collected for research purposes and not for medical diagnostics. Participants will understand that if the physician detects no unexpected findings, this is not an indication that there are no abnormal conditions. Participants will also be informed that in case of an unexpected finding the physician will consult a colleague medical specialist (radiologist, neurologist, cardiologist, etc) and the case will be discussed. In case the physician and the contacted specialist find follow-up necessary, the participant's general practitioner (GP, "huisarts") will be contacted within 3 weeks after the scan. The participant will receive notice that the GP was contacted; the GP will inform the participant about the findings. Because of possible scientific consequences the researcher will also be informed about unexpected findings. The participant needs to agree prior to the experiment that they agree to be contacted by their GP in the case of unexpected findings.

All standard anatomical images (i.e. localizer images) are routinely reviewed by a neuroradiologist. FMRI studies are not given a clinical interpretation. In the event that a significant abnormality is detected, a recommendation to seek further medical consultation will be made. However, it is stressed that the MRI evaluation performed for these studies does not represent a complete clinical MRI evaluation, and it is not being performed for clinical diagnostic purposes.

9. STATISTICAL ANALYSIS

9.1 Primary study parameter(s)

Behavioral analyses

This study will mainly generate qualitative continuous data. For behavioural measures and questionnaires (e.g. on personality and peer relationships), mean scores will be calculated by averaging over trials or items, respectively. In the case of missing data, the researchers will first try to recover the values by contacting the participants, if possible. If data is missing completely at random, multiple imputation will be used in cases where more than 10% of data are missing. Otherwise, listwise deletion will be used. Whether data is missing completely at random, or not at random, missing data and how it was handled will be reported in all publications that result from this study.

Regarding the behavioural data, participant's choices from each condition

will be acquired, as well as reaction times, and these will be compared using 1) regression analyses with age as predictor and 2) repeated measures ANOVAs with condition as a within subjects' variable, and age group as a between-subjects variable. Additionally, mediation models will be used to examine whether brain development or participant characteristics mediate the relationship between for example age and prosocial behavior. Moreover, moderation analyses (i.e., interactions in regression analyses or ANOVAs) will be used to examine whether for example the strength of the relationship between parenting styles and prosocial behavior differs as a function of age. If necessary, analyses will be corrected for multiple comparisons using Bonferroni corrections. If assumptions of parametric analyses cannot be met, non-parametric analyses will be performed.

fMRI analyses

fMRI data will be preprocessed using SPM8 (Welcome Department of Cognitive Neurology, London). Images will be corrected for differences in timing of slice acquisition, followed by rigid body motion correction. Structural and functional volumes will be spatially normalized to T1 and EPI templates, respectively. Translational movement parameters may never exceed 1 voxel (<3 mm) in any direction for any subject or scan. The normalization algorithm uses a 12-parameter affine transform together with a nonlinear transformation involving cosine basis functions and resamples the volumes to 3mm cubic voxels. Templates will be based on the MNI305 stereotaxic space (Cocosco et al., 1997), an approximation of Talairach space (Talairach and Tournoux, 1988). Functional volumes will be spatially smoothed with an 8mm FWHM isotropic Gaussian kernel. Statistical analyses will be performed on individual subjects data using the general linear model in SPM8. The fMRI time series data will be modeled by a series of events convolved with a canonical hemodynamic response function. The trial functions will be used as covariates in a general linear model, along with a basic set of cosine functions that high-pass filtered the data, and a covariate for session effects. The least-squares parameter estimates of height of the best-fitting canonical HRF for each condition will be used in pair-wise contrasts. The resulting contrast images, computed on a subject-by-subject basis, will be submitted to group analyses. Task related responses will be considered significant if they consist of at least 10 contiguous voxels that exceeded a threshold $p<0.05$ (FDR-corrected; (Genovese et al., 2002). Analyses will also be performed with less stringent thresholds, $p<0.001$ uncorrected, using cluster corrected (FDR, $p<.05$).

One possible caveat is the use of a single canonical hemodynamic response function (HRF) to model the fMRI data for all age groups. Reassuringly, recent findings suggest

that the hemodynamic response function does not differ between children and young adults (Kang et al., 2003;Thomason et al., 2005). Moreover, a review of the pediatric neuroimaging literature reveals that children show greater activation than adults in some regions and less activation in others, and that these regional differences are highly task-dependent, arguing against systematic age differences in the fit of the canonical HRF.

ROI analyses will be performed on functionally defined cortical and subcortical regions with the MarsBar Toolbox in SPM8 (Brett et al., 2002); available at marsbar.sourceforce.net). For all experiments, ROIs will be identified based on general contrasts, such as all correctly performed trials relative to fixation. Dependent measures will be compared using multivariate ANOVAs using condition and Age group as a between-subjects variable. In addition, regression analyses will be performed to examine whether age or other individual differences scores can predict differences in brain activation.

DTI; analyses of anatomical connectivity

Preprocessing of DTI data will be carried out using FMRIB's Diffusion Toolbox (FDT) from FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). DTI shows in vivo the freedom of diffusion, i.e. Brownian motion, of water molecules in the brain. The freedom of diffusion can severely be restricted by the isolating qualities of different neural tissue types. That is, in the cerebro-spinal fluid (CSF) water molecules can move freely in either direction. However, the isolating properties of myelin cause the molecules to move in just one direction. By measuring this strength of restrictedness, i.e. fractional anisotropy (FA), it is thus possible to localize white matter pathways and make inferences on their integrity. In this study, fractional anisotropy will be compared between the age groups using tract-based spatial statistics (TBSS) in FSL (Smith et al., 2006). In addition, we will examine whether there are regions in which FA values are correlated with cognitive control test scores by entering these values as covariates in the TBSS analysis (Olesen et al., 2003).

Structural MRI analyses

To measure differences in local concentrations of gray matter, voxel-based morphometry (VBM) will be used (Ashburner & Friston, 2001; Ashburner & Friston, 2000; Mechelli et al., 2005; Kennedy et al., 2008). This technique involves a voxel-wise comparison of gray and white matter concentration between groups of subjects. In the present study, first, high-resolution images will be spatially normalized into stereotactic space by registering each of the images to the same template image. Then, the spatially normalized images

will be segmented using a cluster analysis technique to identify voxels belonging to particular tissue types. Modulation, which multiplies the gray (and white) matter voxels by the Jacobians derived from spatial normalization, will be used to preserve the amount of gray (and white) matter in each voxel. To reduce confounds caused by individual differences in gyral anatomy, the gray and white matter images will be smoothed by using an isotropic Gaussian kernel of FWHM 8 mm. Statistical tests, based on t tests and the general linear model will be used to locate regionally specific differences in gray matter between age groups. In addition, cognitive control test scores and age will be entered in the VBM analysis in order to estimate how gray matter density correlates with cognitive control.

Cortical surface reconstruction and cortical thickness measurement

Reconstruction of the cortical surfaces will be performed using the FreeSurfer toolkit (which is freely available to the research community through the website http://www.surfer.nmr.mgh.harvard. edu/). This suite of methods was initially proposed in 1999 (Dale et al., 1999; Fischl et al., 1999a) and has undergone several important improvements over the years (Fischl & Dale, 2000; Fischl et al., 2002; Fischl et al., 1999b; Segonne et al., 2004). Cortical thickness estimates in this study will be computed as follows. For each subject, cortical thickness will be estimated as the distance in millimeters between the white matter (gray/white boundary) and gray matter (gray/cerebrospinal fluid boundary) cortical surface (Fischl and Dale, 2000). The white and gray cortical surfaces will be reconstructed from the raw unaligned images in native space, as implemented in the FreeSurfer software package (Dale et al., 1999; Fischl & Dale, 2000). The reconstruction process will be supervised and corrected when necessary by an operator blind to the subject's identity. The reconstruction procedure will be repeated until accurate white and gray surfaces were obtained. For each subject, the total intracranial volume will also be estimated as described by (Buckner et al., 2004). The reconstructed surfaces enabled calculation of cortical thickness, surface area, and regional gray matter volume at every vertex (i.e., surface point) with methods developed by Fischl and Dale (Fischl & Dale, 2000). For each subject, the cortical surface will be separated into gyri and sulci by thresholding the cortical surface curvature values. The curvature threshold will be fixed at 0, the surface point of inflexion between gyri and sulci. This is the point where the cortical surface passed from convex to concave or vice versa. Effectively, sulci contain both sulcal wall and fundus. For analyses of cortical thickness, surface, and volume, age will be entered a priori as a covariate, because age seems to be related to these SBM variables (Shaw et al., 2008). In analyses of surface and volume, we will also control for intracranial volume. For cortical thickness, the relationship with

intracranial volume is not clear. Therefore analyses will be performed with and without including intracranial volume as a covariate. Cortical thickness maps at every vertex are compared and correlated with cognitive control scores by means of a General Linear Model.

Analyses of hormones

Free (bioavailable) testosterone, DHEAS, estradiol, cortisol and oxytocin will be determined in hair and saliva samples using immunoassays and tandem mass spectroscopy.

Statistical models

In order to determine the developmental trajectories of behavior, functional and structural brain development, and psycho-physiological variables multilevel models of change will be used, as these permit the inclusion of multiple measurements per person (Shaw et al., 2008). For each dependent measure a mixed-effects polynominal regression model, testing for cubic, quadratic, or linear age effects, will be fitted. The cubic effect is considered most complex, followed by quadratic, which is held more complex than linear models. This approach allows the classification of each dependent measure as being best predicted by cubic, quadratic and linear age effects. These effects are predicted based on structural brain development studies (Shaw et al., 2008). In addition, several additional measurements will be obtained and related to functional brain activation and structural brain measures, such as demographics, average school grades, peer network, IQ, and social-economical status.

This approach will be applied for the behavioral data, the functional data, the hormone level data, and the structural cortical (white matter microstructure, grey matter thickness, volume) and subcortical (volume size) data with two main goals: (1) to examine the growth curve for cognitive versus emotional and social-emotional functional brain development and examine whether these are differences in tempo and shape of the curves, and (2) to examine whether behavior, hormone levels and structural brain development can predict functional brain development.

9.2 Secondary study parameter(s) : N/A

9.3 Other study parameters: N/A

9.4 Interim analysis: N/A

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza Brazil, 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and Protocol ethiek voor wetenschappelijk onderzoek van de Faculteit Sociale Wetenschappen RU Leiden, december 1997.

10.2 Recruitment and consent

Participants will be recruited through information posted on the Brain and Development lab website www.brainanddevelopmentlab.nl and a recruitment website especially focussed on children/youth: http://www.juniorhersenen.nl. These websites will be used to recruit adult participants from universities and other institutions in the Leiden area.

The developmental population will be recruited in collaboration with local schools. Participation via schools is a procedure which has previously been successfully used in prior longitudinal studies. The researchers are aware of the additional requirements when collaborating with schools. Even though the recruitment will take place via school, participants need to actively sign up for the MRI part of the study. Parents and children will be given written information about the study (see appendix E). The parents and the child will contact the research team to express their interest for participation. All advertisements will indicate that participation in the experiment is completely voluntary. A similar procedure has previously been successfully used in protocol nr. P10.191, nr. P10.193 and nr. P10.192., which was approved by the CCMO, and protocol nr. P05.118 and P16.014, which were approved by the CME in Leiden.

Once participants have contacted the research team about their willingness to participate in the study, the general procedures used during the study will be explained, and participants will be screened for any counter-indications to MRI (including a history of psychiatric or neurological disorders). Participants will additionally be screened for psychiatric disorders on the day of participation using the CBCL and SCL (see 8.3. Study procedure). Only those individuals that score within the normal range will be included in the analysis. Written informed consent will be obtained by the investigator from the subject or the parent/legal guardian of the subject (if the subject is under 18 years of age), or both (in case the subject is between 12 and 18 years of age). The consent form will be kept inside a locked filing cabinet accessible only to lab personnel. At the time of consent, any remaining questions the subject or parents may have will be answered.

10.3 Objection by minors or incapacitated subjects (if applicable)

The Nederlands Vereniging voor Kindergeneeskunde (NVK) code of conduct; Gedragscode verzet bij minderjarigen die deelnemen aan medisch-wetenschappelijk onderzoek will be applied to this study.

10.4 Benefits and risks assessment, group relatedness

Behavioral testing: There are no risks associated with behavioral testing except the occasional possibility of some frustration with poor performance or fatigue. There will be breaks between tests in order to allow participants to rest and prevent poor performance due to fatigue. Testing will stop if a subject displays frustration or appears tired.

FMRI testing: There are no known risks associated with participating in an fMRI study. This is a noninvasive technique involving no catheterizations or introduction of exogenous tracers. Numerous human subjects have undergone magnetic resonance studies without apparent harmful consequences. Radiofrequency power levels and gradient switching times used in these studies are within the FDA approved ranges. Some people become claustrophobic while inside the scanner, and in these cases the study will be terminated immediately at the subject's request. The only absolute contraindications to MRI studies are the presence of intracranial or intraocular metal, or a pacemaker. Relative contraindications include pregnancy and claustrophobia. Subjects who may be pregnant, who may have metallic foreign bodies in the eyes or head, or who have cardiac pacemakers will be excluded because of potential contraindications of MRI in such subjects.

Potential Benefits of the Proposed Research to the Participants and Others: Although there is no direct benefit to the participants from this proposed research, there are greater benefits to society from the potential knowledge gained from this study, as described in the next section.

Importance of the Knowledge to be Gained: This study aims to acquire knowledge about the development of prosocial behavior in adolescence. This knowledge about typical development is critical to aid in the understanding of cases of atypical development, as seen in children with conduct disorder, but also in eventually fostering prosocial behavior and the associated benefits in adolescents.

Risk-to-Benefit Ratio: The importance of the benefits gained from this research far outweighs the minimal risks involved.

Protection Against Risk: Participants will be protected against any MRI procedural risks via a thorough pre-screening process. Information obtained from the studies in the research plan will be strictly confidential, except as required by law, but will be made available to the subject and his/her physician in response to a specific request from the subject. There will be no personal identification of subjects in scientific communications. Data will be stored in a confidential manner both through the use of a numbering system (a number will be assigned to the data from a given subject instead of the subject's name) and through the security of the files and computer systems.

Inclusion of Children

Children, adolescents and young adults (9 - 18 years) will be included in this study. Children are included only when they are 9 years or older. The ages targeted in this study are separated enough to show significant developmental changes with respect to the construct of prosocial behavior.

Prof. Crone has extensive experience conducting fMRI research on children at the LUMC (see resumes at www.brainanddevelopmentlab.nl) and is familiar with the added considerations involved in testing children as compared with adults. Prof. Crone will be responsible for training other study personnel to be fully aware of the special needs of children.

The Leiden University Medical Center (LUMC) has been equipped with facilities especially focused on children. A mock scanner is installed at the radiology department that has been used successfully in the past years in several studies which included children and adolescents ages 8-18. All children and adolescents who have participated so far have indicated they enjoyed the experience, and that they would be interested in participating again. In the present project all participants will visit and lie in the mock scanner and will be given opportunity to ask questions about the mock and real MRI scanner.

All minors were, and will be given the opportunity to adjust to the MRI environment – which involves lying still on the scanner bed, watching the screen through a mirror attached to the head coil, and hearing the loud radio-frequency pulses – before participating in the study. All participants will lie in the mock scanner and practice performing the task prior to the experiment, care will be taken to ensure participants that at that point and at any time during the study they have the opportunity to decide not to participate.

It is extremely important that the minors in this project participate on a voluntary basis. Therefore, the researchers will ensure that the 'Code Verzet' or in English 'Code of conduct relating to expressions of objection by minors participating in medical research' will be properly and meticulously applied. In order to achieve this, all regulations in this code will be followed, with special attention for emphasizing to both parents and children that their participation is absolutely voluntary and that they can stop at any moment if they do no longer want to participate. In consultancy with the parents it will be established in which way children are likely to express any objection to their participation. Any signs of objection or behaviour that is not is within the bounds normally associated with the child will be checked for throughout the study. If children object to their participation the child's participation will immediately be discontinued.

10.5 Compensation for injury

Although the proposed research is without risk, for everyone who participates in this study there is an insurance policy. The insurance covers damages resulting from the research. This applies to damages that comes up during the investigation and within four years after the end of the study. See Appendix G for the insured amounts, exceptions and details of the insurer

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.6 Incentives

Participants will receive a small present package (such as a pen, usb-stick or other gadgets) for participating in the study, as well as monetary compensation that will be paid to the parents and is intended for travel compensation

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

Data will be handled confidentially and anonymous. A separate subject identification code list will be used to link the data to the subject. The code is based on the first letter of the study (e.g. P for Prosocial and the test number (P001, P002, P003, etc.). The subject identification code list will be safeguarded by the principal investigator. The personal data will be handled according to the Dutch Personal Data Protection Act (Wet Bescherming Persoonsgegevens, Wbp)

11.2 Monitoring and Quality Assurance: N/A

11.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

Non-substantial amendments will not be notified to the accredited METC and the competent authority (CA), but will be recorded and filed by the sponsor.

11.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient’s last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.6 Public disclosure and publication policy: N/A

12. STRUCTURED RISK ANALYSIS: N/A

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