Like me, or else: Nature, nurture and neural mechanisms of social emotion regulation in childhood

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CHAPTER NINE

General discussion

Summary

The is thesis had the goal to provide a better understanding of why some children are more sensitive to social evaluation than others, a question that is currently more urgent than ever, given that young individuals connect not only through personal interactions but also through online communication. This thesis examined this question from a neurocognitive development perspective and incorporated both behavioral genetic modeling as well as longitudinal analyses. Neurodevelopmental models suggest that social emotional regulation can be partly explained by protracted development of subcortical and prefrontal cortex regions, as well as their connections (Nelson *et al.*, 2005; Casey *et al.*, 2008; Casey, 2015; Nelson *et al.*, 2016). These models focus mostly on adolescence, the transition period between childhood and adulthood, whereas childhood is a relatively unexplored phase in experimental neuroimaging research. Nevertheless, during childhood rapid changes in executive functioning occur (Luna *et al.*, 2004; Zelazo and Carlson, 2012; Peters *et al.*, 2016) and the first long lasting friendships emerge during this time (Berndt, 2004).

Social emotion regulation is an important factor in developing and maintaining these social relations. Social emotion regulation consists of processing social information (such as peer feedback) and regulating subsequent emotions and behaviors (such as aggression). A broad range of literature has shown that social rejection can result in behavioral aggression (Twenge *et al.*, 2001; Dodge *et al.*, 2003; Leary *et al.*, 2006; Nesdale and Lambert, 2007; Nesdale and Duffy, 2011; Chester *et al.*, 2014), but little is known about the underlying mechanisms of social rejection related aggression. This thesis aimed to fill this gap by investigating the nature, nurture, and neural mechanisms underlying social emotion regulation in childhood.

Testing the Social Network Aggression Task

In order to gain a better understanding of the underlying mechanisms of responses to social acceptance and rejection, I co-designed a novel experimental paradigm that is suitable to combine with neuroimaging. In the Social Network Aggression Task (SNAT) participants view pictures of peers that provide positive, neutral or negative feedback to the participant's profile. In addition to neural activation related to social acceptance and rejection, this paradigm enables studying regions that signal for general social salience, by contrasting both positive and negative feedback to a neutral social feedback condition. To study individual differences in behavioral responses towards social evaluation, we included a retaliation component to the SNAT. After viewing the social feedback, participants could blast a loud noise towards the peer, which was used as an index of aggression.

A crucial first step in understanding social evaluation processing in childhood is to detect robust behavioral patterns and neural signals that are related to processing social feedback. Therefore, in chapter 2 I used a meta-analytic approach to examine behavioral and neural correlates of social evaluation processing in seven-to-eleven-year-old children. I used three different samples: a pilot sample (n=19), a test sample (n=28), and a replication sample (n=27). The results showed that the SNAT revealed robust and reliable behavioral results with negative social feedback resulting in the highest levels of behavioral aggression. Moreover, meta-analyses on predefined brain regions of interest (ROIs) revealed that negative social feedback resulted in more neural activation in the amygdala (compared to positive feedback), the anterior insula (AI) and the anterior cingulate cortex gyrus (ACCg) (compared to neutral feedback). Exploratory whole brain analyses demonstrated heightened activation in the medial prefrontal cortex (MPFC) after negative relative to neutral social feedback. These findings show that the SNAT is a reliable paradigm for the investigation of social evaluation processing and aggression in children, and indicate that this paradigm is feasible for use in larger and longitudinal developmental studies.

Next, in chapter 3, I investigated the neural processes of social evaluation in adults. The aims of this study were three-fold: (1) to disentangle neural signals of positive and negative social feedback, (2) to examine aggressive responses toward the person signaling negative social feedback and (3) to test whether dorsolateral prefrontal cortex (DLPFC) activity was related to aggression regulation after experiencing negative social feedback, based on prior studies with comparable paradigms (Riva *et al.*, 2015). The DLPFC is a region often found implicated in behavioral control (Casey, 2015; Crone and Steinbeis, 2017). In line with the meta-analytical results of chapter 2, I found that negative social feedback was related to applying a longer noise blast toward the peer. At the neural level, conjunction analyses showed that both negative and positive social feedback resulted in increased activity in the ACCg and the bilateral AI, suggesting that these two regions generally respond to socially salient feedback, with no significant differentiation between negative and positive feedback. Neural activation that was specific for positive feedback was located in the striatum and the ventral MPFC, whereas there was no specific significant activation after negative (versus positive) social feedback. Brain-behavioral analyses, however, showed that increased DLPFC activity after negative social feedback was related to more aggression regulation. These results imply that individuals who show stronger activation in the DLPFC after negative social feedback may be better able to regulate social emotions and behavioral impulses.

Social emotion regulation in childhood

After verifying the experimental paradigm in children and adults, the next step was to examine to what extent individual variation in social evaluation was

explained by genetic and environmental factors. Some children might be more sensitive to social evaluation due to genetic predisposition, but likewise, children might be more prone to retaliation due to environmental influences such as violent video games (Konijn *et al.*, 2007). Unraveling these contributions is important as little is known about the genetic and environmental influences on brain responses to social feedback and regulatory responses. Behavioral genetic modeling can estimate the proportion of variance that is explained by additive genetics (A), common environment (C) and unique environment and measurement error (E).

In chapter 4, I used behavioral genetic modeling to investigate the heritability of social feedback processing and subsequent aggression in middle childhood (ages 7-9-years). Behavioral genetic modeling revealed that aggression following negative feedback was influenced by genetic as well as shared and unique environmental influences. Experimental neuroimaging analyses of a large childhood sample (N=512) showed again that the AI and ACCg responded to both positive and negative feedback (see also chapter 2 and 3), showing this social salience network is already present in childhood. Similar to what was observed in the pilot-test-replication study (chapter 2); positive feedback resulted in increased activation in caudate, supplementary motor cortex (SMA), as well as in the DLPFC. In this study I further observed that the MPFC and inferior frontal gyrus (IFG) were more strongly activated after negative feedback. To test relations with behavior in more detail, post-hoc analyses were performed using the significant whole brain clusters as ROIs. These analyses demonstrated that decreased SMA and DLPFC activation after negative feedback (relative to positive) was associated with more aggressive behavior after negative feedback. Thus, similar to what was observed in adults in chapter 3, in children the DLPFC was an important region for aggression regulation. Moreover, genetic modeling showed that 13%–14% of the variance in DLPFC activity was explained by genetics. These results suggest that the processing of social feedback is partly explained by genetic factors. Moreover, whereas the social salience network seemed to be in place already in middle childhood, the aggression regulation mechanism was less pronounced in middle childhood than in adults, which might suggest that this network is still developing during childhood. A final intriguing finding in chapter 4 was that the behavioral response to aggression (i.e., noise blast) was influenced by shared environment factors. Together, these findings set the stage to examine how brain responses (influenced by genetic factors) and behavior (influenced by shared environment factors) change over time.

Chapter 5 set out to test exactly this question, that is, to test developmental changes in aggression regulation and the underlying neural mechanisms using a longitudinal design. In this chapter I examined how changes in neural activity across childhood were related to change in behavioral development. For this purpose 492 same-sex twins (246 families of the original 256 families) underwent two fMRI sessions across the transition from middle

childhood (7-9 years) to late childhood (9-11 years). Results showed that behavioral aggression after social evaluation decreased over time, and this decrease was most pronounced for aggression after positive and neutral social feedback. Confirmatory ROI analyses showed that neural activity in the AI, MPFC and DLPFC increased across childhood, whereas activity in the IFG did not show developmental change. Moreover, increased activity in AI was correlated with more aggression, whereas increased activity in DLPFC was correlated with less aggression. Whole brain-behavior analyses confirmed that bilateral DLPFC activity was correlated with less subsequent aggression following negative social feedback. Finally, longitudinal comparisons revealed that a larger increase in DLPFC activity across childhood was related to a larger decrease in behavioral aggression after negative social feedback over time. These results provide insights on how the developing brain processes social feedback and suggest that the DLPFC serves as an emotion regulation mechanism when dealing with negative social feedback. The results provide a window for understanding individual differences in these developmental trajectories, showing that some children develop stronger regulation skills already in childhood.

Functional architecture of the childhood brain

Previous neurodevelopmental studies and theoretical frameworks have suggested that social emotion regulation might rely on a network of integrated connections between limbic/subcortical and cortical brain regions (Casey, 2015). Most prior studies focused on adolescence or included small samples of children and therefore little is known about functional brain connectivity in childhood. To overcome this gap in knowledge, in chapter 6 I investigated the robustness of findings regarding subcortical-PFC functional brain connectivity in childhood, and the heritability of these connections in 7-to-9-year-old twins. I specifically focused on two key subcortical structures: the ventral striatum (VS) and the amygdala. Reassuringly, I observed strongly replicable brain connectivity patterns over two genetically independent samples of 7- to-9-year-old children, both in the whole brain seed-based analyses and in the post-hoc ROI analyses. Behavioral genetic analyses revealed that VS and amygdala connectivity showed distinct influences of genetics and the environment. VS-PFC connections were best described by genetic and unique environmental factors, whereas amygdala-PFC connectivity was mainly explained by environmental influences (both shared and unique). Similarities were also found: connectivity between the ventral ACC and both subcortical regions showed influences of shared environment, while connectivity with the orbitofrontal cortex (OFC) showed stronger evidence for heritability. Together, this study provides the first evidence for a comprehensive analysis of genetic and environmental effects on subcortical-prefrontal cortex interactions in childhood. The findings demonstrate the need to understand not

only the development of these networks, but also how the environment shapes the maturation of these connections.

Neuroimaging in childhood: Pitfalls and possibilities

With the emergence of functional neuroimaging only two decades ago, the field of developmental cognitive neuroscience can still be considered relatively young and acquisition methods and analysis techniques are rapidly improving. Several prior developmental neuroimaging findings have been called into question after studies showed that these findings were largely influenced by age-related differences in head motion (Satterthwaite *et al.*, 2013), highlighting the need for an in-depth investigation of factors that can influence scan quality in children. In chapter 7 I therefore provide an overview of MRI scan quantity and quality in a large developmental twin sample and investigated the genetic and environmental influences on head motion. Overall, scan quantity was high (88% of participants completed all runs), while scan quality decreased with increasing session length. Scanner related distress was negatively associated with scan quantity, but not with scan quality. In line with previous studies, behavioral genetic analyses showed that genetics explained part of the variation in head motion, with heritability estimates of 29-65%. Additionally, the results revealed that subtle head motion - after exclusion of excessive head motion- showed lower heritability estimates (0–14%), indicating that findings of motion-corrected and quality-controlled MRI data are less confounded by genetic factors. Moreover, shared environmental influences played a larger role (15–33%) in the variation in quality-controlled head motion, suggesting that head motion can be influenced by participant instruction and age-appropriate scanner adjustments. This is specifically important for neuroimaging studies across different age-ranges, as this can minimize the confounding factor of age-related differences in head motion on findings regarding brain development.

Brain connectivity as predictor of emotion regulation

As was explained in the section on neurocognitive development models, the ability to regulate emotions and control impulses increases considerably during adolescence, the transition phase between childhood and adulthood. In chapter 8 I tested the hypothesis that this form of emotion regulation is driven by increased maturation of frontostriatal circuitry using a fiber-tracking approach combined with longitudinal imaging. Given the novelty of this approach, here I made use of a classic and often used paradigm to study impulse control; the delay discounting paradigm (Peper *et al.*, 2013). The delay discounting task estimates the preference to choose for a direct small reward over a delayed larger reward. In total, 192 healthy volunteers between 8 and 26 years underwent diffusion tensor imaging scanning and completed the delay discounting task twice,

separated by a 2-year interval. This sample was part of the 3-wave longitudinal Braintime study (van Duijvenvoorde *et al.*, 2016b). First, I examined linear and non-linear development of both brain connectivity and behavior. The development of delay of gratification showed a quadratic trajectory, with a steep increase during late childhood and the peak in late adolescence. Structural brain connectivity showed cubic relations across development, with the most pronounced changes during late childhood and early adolescence. Moreover, age related increases in the preference for delayed rewards (i.e., less impulsive choice) were significantly dependent on a better quality of connections between the PFC and striatum. The longitudinal analysis revealed that stronger connectivity between striatum and PFC predicted less impulsive choices 2 years later, indicating that brain maturation precedes emotion regulation and behavioral outcomes. These findings fit well with neurocognitive models suggesting that striatum-prefrontal cortex maturation is an important factor contributing to the development of emotion regulation (Casey, 2015; Nelson *et al.*, 2016).

Discussion

Taken together, the studies described in this thesis revealed several important findings. First, using the Social Network Aggression Task I was able to disentangle between neural activation that was specific for social rejection and social acceptance, and activity that was related to general social salience. Second, by including a retaliation component to the paradigm, I showed how individual differences in aggression regulation were related to differences in neural activation of the DLPFC. Third, by combining findings of task-based functional MRI with both functional and structural connectivity analyses, I gathered knowledge on the development of social emotion regulation and shed light on the important neural development that takes place during childhood. These three main outcomes are discussed in detail below and suggestions for a novel theoretical framework are provided.

Social pain, social gain and general social signaling

Prior studies on social evaluation processing have suggested that the ACC and AI might signal for social pain, as these regions showed increased neural activation after social rejection (Eisenberger and Lieberman, 2004; Kross *et al.*, 2011; Rotge *et al.*, 2015). However, several researchers have questioned this hypothesis as they reported increased activation of the ACC also in relation to expectancy violation (Somerville *et al.*, 2006; Cheng *et al.*, 2019), indicating these regions might signal for social salience in general (Dalgleish *et al.*, 2017). The Social

Network Aggression Task is the first social evaluation paradigm to experimentally disentangle neural activation for social rejection and social salience, by contrasting positive and negative social feedback to a neutral condition. In order to provide a comprehensive overview of the findings from the SNAT paradigm, I conducted a meta-analysis on the neural activation after general social salience (positive and negative feedback vs. neutral feedback), social rejection (negative vs. positive feedback) and social acceptance (positive vs. negative feedback). For this analyses I used GingerALE (Eickhoff *et al.*, 2009; Eickhoff *et al.*, 2012; Turkeltaub *et al.*, 2012), a Brainmap application that is based on activation likelihood estimation, with p<.005 and a minimal volume threshold of 300 mm2. Meta-analytical results are based on the findings of adults (chapter 3, table S1 and S3), middle childhood (chapter 4, table 3) and late childhood (chapter 5, table S6) and show distinct neural activation for social rejection and social acceptance, and additionally reveal a network of brain regions that are sensitive to general social salience, see **Table 1** and **Figure 1**.

Social rejection resulted in increased neural activation in the bilateral IFG, the MPFC, and visual regions in the occipital lobe, including the cuneus (**Table 1**, **Figure 1**). Previous studies often failed to find significant neural activation after negative social feedback (Gunther Moor *et al.*, 2010b; Guyer *et al.*, 2012) which could be related to low statistical power, as these studies often used small sample sizes (Mumford and Nichols, 2008; Button *et al.*, 2013). In chapter 3 of this thesis I also did not report significant activation after social rejection using a smaller sample size (n=30) in an adult sample. However, in the studies with large samples and strong statistical power (chapter 4 and 5) I consistently report strong activation in the IFG and MPFC in childhood. The MPFC has shown to play an important role in social cognition and behavior (Blakemore, 2008; Adolphs, 2009) and is specifically implicated when thinking about others (Apps *et al.*, 2016; Lee and Seo, 2016). Receiving negative social feedback may leave the children wondering what the other might have thought about them (Gallagher and Frith, 2003). Indeed, the social information processing network (SIPN) suggests that the MPFC is part of the "cognitive-regulatory node" were the mental states of others are perceived before inhibition of pre-potent responses are regulated by the lateral PFC (Nelson *et al.*, 2005; Nelson *et al.*, 2016). This corresponds to the MPFC specifically being activated after social rejection, as this might result in a stronger need for social emotion regulation than feedback leading to social acceptance.

Meta-analytical results showed that social acceptance specifically activated regions in the DLPFC, the SMA, and visual regions in the occipital lobe (**Table 1**), consistent with prior studies on social evaluation processing (Gunther Moor *et al.*, 2010b; Guyer *et al.*, 2012). The chosen GingerALE setting of clusters > 300 mm2 limits the possibility of finding meta-analytical activation in small regions such as the striatum, however, I did report significant activation in the caudate in both adults (chapter 3) and children (chapter 4). The SMA and DLPFC

have been related to motor planning and behavioral control (Casey, 2015; Riva *et al.*, 2015) and neural activation in these regions might be related to the retaliation component of the SNAT paradigm. That is, participants might like the peers that provided positive feedback and therefore be intrinsically motivated to release the button as soon as possible, resulting in increased activation in the SMA and DLPFC. Indeed, the behavioral results showed that participants liked social acceptance the most and the rewarding value of positive feedback was also depicted in increased striatum activation (Sescousse *et al.*, 2013). Increased striatal activation after positive feedback has been reported by previous social evaluation studies (Davey *et al.*, 2010; Gunther Moor *et al.*, 2010b; Guyer *et al.*, 2012) and fits well with the SIPN model that highlights the importance of the "affective node" (including striatal regions) in the processing of social stimuli (Nelson *et al.*, 2005; Nelson *et al.*, 2016).

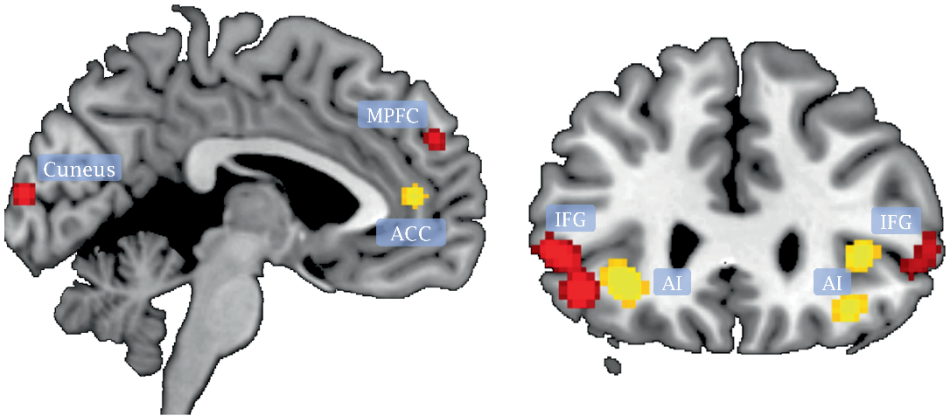

Figure 1. *Meta-analytic activation maps for Social Network Aggression Task studies of chapters 3, 4 and 5. Neural activation for social rejection (negative > positive feedback) depicted in red. Neural activation for general social salience (positive and negative > neutral feedback) depicted in yellow. Meta-analyses were conducted using GingerALE with p<.005 and volume > 300 mm2.*

Using the SNAT, I experimentally showed that there is a neural network sensitive for general social salience, irrespective of its valence. Both positive and negative social feedback resulted in increased neural activation in the ACCg, bilateral AI, medial frontal gyrus and visual regions in the occipital lobe (**Figure 1**, **Table 1**). These findings fit with the literature suggesting that the ACC and AI signal for social salience in general (Somerville *et al.*, 2006; Dalgleish *et al.*, 2017; Cheng *et al.*, 2019). These findings add to previous theoretical models of social information processing which indicated the fusiform face area as an important social detection mechanism (Nelson *et al.*, 2005; Nelson *et al.*, 2016), by showing

that the ACC and AI are also important in the detection and signaling of social relevant information. Moreover, the social salience networks reported in adults (chapter 3), middle childhood (chapter 4) and late childhood (chapter 5) show remarkable resemblances, indicating this might be a core social motivational mechanism in humans. This highlights the importance of incorporating childhood neurodevelopmental changes into theoretical frameworks, as social processing networks are already active during childhood. Moreover, chapter 5 describes how activation in the AI was related to behavioral aggression, and future studies should further explore whether individual differences in neural activation of the social salience network are related to individual differences in sensitivity to social evaluation. By taking real-life social interactions into account, future studies might be able to examine whether individual differences in sensitivity to social evaluations are a cause or an effect of individual differences in social (offline or online) interactions.

Aggression regulation following social feedback

Previous theoretical models of social emotion regulation have suggested that the lateral PFC is important for top down control over affective-motivational subcortical regions (Nelson *et al.*, 2005; Casey *et al.*, 2008; Casey, 2015; Nelson *et al.*, 2016). By including a retaliation component to the Social Network Aggression Task, I was able to directly test how individual differences in social emotion regulation were related to neural activation in the DLPFC. Consistent with prior experimental studies (Riva *et al.*, 2015), chapter 3 revealed that increased activation in the DLPFC after social rejection was related to less subsequent aggression in adults, suggesting that these individuals were more successful at regulating their behavioral aggression. Region of interest analyses of the DLPFC in a middle childhood sample (chapter 4) provided some indications of an aggression regulation network, but this was not strong enough to be depicted using whole brain-behavior analyses. When examining these same children two years later - now during late childhood - there was a significant association between brain and behavior. Similarly to adults, increased neural activation in the DLPFC was related to less behavioral aggression after negative social feedback. Importantly, the children who displayed the largest developmental increases in DLPFC activity across childhood also displayed the largest changes in social emotion regulation. These findings add to previous studies that suggested that the DLPFC is an important region for cool (non-emotional) cognitive control (Luna *et al.*, 2004; Luna *et al.*, 2010; Crone and Steinbeis, 2017) by showing that the DLPFC is also important in controlling hot emotional control (Zelazo and Carlson, 2012; Welsh and Peterson, 2014). Moreover, the results provide evidence for developmental models of social emotion regulation (Nelson *et al.*, 2005; Casey *et al.*, 2008; Casey, 2015; Nelson *et al.*, 2016) in such a way that they confirm that the DLPFC serves as a regulatory

mechanism and is related to behavioral outcomes. However, these models specifically focused on adolescent brain development, whereas the findings of this thesis show that important changes in this neural network occur during childhood. Theoretical perspectives based on behavioral studies have suggested that the development of emotion regulation is closely related to the development of cognitive control (Diamond, 2013) and experimental studies have shown that cognitive control development accelerates during childhood (Luna *et al.*, 2004; Zelazo and Carlson, 2012; Peters *et al.*, 2016). The current thesis provides direct links between maturation of cognitive control (DLPFC) regions and individual differences in social emotion regulation. This was shown in a specific age range (7-9-year old to 9-11-year old), to provide a detailed analysis of changes in childhood. The results provide a window for understanding individual differences in these developmental trajectories, showing that some children develop better regulation skills already in childhood. Future research should examine developmental changes in a longer time window by including more measurement points, which allows disentangling general developmental patterns from individual differences in growth trajectories.

Childhood: A window of opportunity

As children grow older and move towards adolescents, they generally receive more autonomy and are less often under adult supervision (Steinberg *et al.*, 1989). In some individuals this results in increased risk taking and sensation seeking, which can have negative consequences such as physical and psychological injury (Steinberg, 2008). To understand individual differences in these behaviors, several neurodevelopmental models have been proposed (see Casey (2015) for an overview), all of which focus on adolescent brain development. The longitudinal analyses across children, adolescents and adults in this thesis (chapter 8), however, showed that structural connectivity between the striatum and the PFC was predictive of behavioral control two years later, providing evidence that brain maturation can forecast future behavioral control. Knowing that brain development precedes behavior (Gabrieli *et al.*, 2015); the foundation for adolescent behavior is thus laid during childhood. The studies in this thesis highlight the importance of incorporating childhood brain development in neuroscientific models by showing that the steepest increases in both behavioral control and subcortical-PFC structural connectivity take place during childhood.

Both empirical studies as well as theoretical models have mostly focused on developmental peaks in brain maturation (Casey *et al.*, 2008; Galvan, 2010; Braams *et al.*, 2015; Peters and Crone, 2017). Although this can be illuminating, I argue that the road towards this peak is more informative when it comes to development. The developmental phase that marks the steep increase preceding the peak is the time in which actual change is taking place. This could possibly

reflect a moment where it is relatively easy to intervene in development. Metaphorically, if a rock is quickly rolling down a hill, one can easily change its course by gently tapping the rock. The faster the stone is rolling, the larger the impact of this small interference will be. However, when the stone has reached the end of the hill, the small tap will no longer have a big impact. As a broad range of studies - including chapters of this thesis- have shown that childhood marks pronounced changes in emotional reactivity (chapter 5; Silvers *et al.* (2012)), cognitive control (Luna *et al.*, 2004; Peters *et al.*, 2016) and structural brain connectivity (chapter 8; Wierenga *et al.* (2018b)). These accelerated changes in brain development could provide a window of opportunity for interventions that can change the course of development with smaller interference compared to later interventions (**Figure 2**).

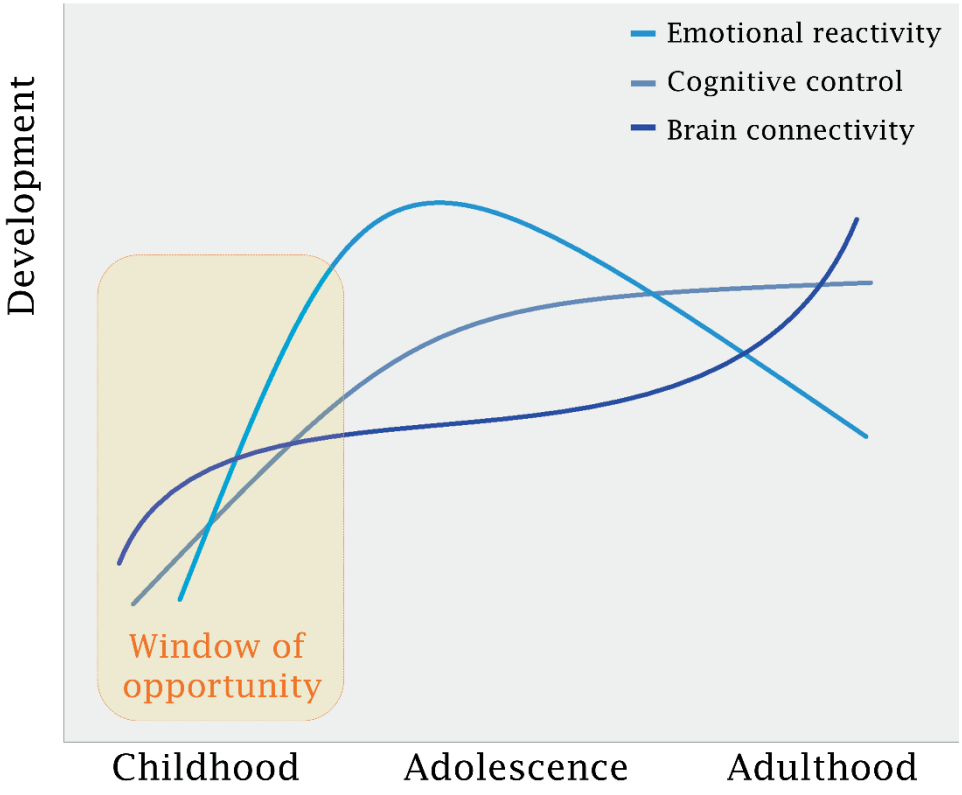

Figure 2. *Childhood as window of opportunity. The steepest increase in emotional reactivity, cognitive control and (structural) brain connectivity are in late childhood, which may reflect a unique window of opportunity in terms of development. Note: data of developmental trajectories are illustrative.*

Methodological Considerations

The studies discussed in this thesis make an important contribution to the literature on the development of social emotion regulation and point to childhood as a possible window of opportunity. Apart from these theoretical implications, there are four methodological considerations that arise from these studies, which are reviewed below.

Two of a kind: Generalizability of twins to singletons

The classical twin design is sometimes referred to as "the perfect natural experiment", as it provides the unique opportunity to tease apart genetic components from environmental influences. Using a twin design can provide important insights in the underlying mechanisms of a psychological construct. An important assumption of these studies is that findings can be generalized to the general (non-twin) population (Moilanen *et al.*, 1999). Although several studies have shown that this is true when it comes to general physical characteristics (i.e., blood pressure or height, (Andrew *et al.*, 2001)), twin-singleton comparisons on psychological constructs are limited. A large longitudinal study in middle and late childhood showed no significant differences between the developmental trajectories of externalizing problems of twins and singletons (Robbers *et al.*, 2010), suggesting that twin findings on behavioral control or emotion regulation might be generalizable. However, when investigating *social* emotion regulation, it is important to keep in mind the unique social buffer that twin-hood might provide (Branje *et al.*, 2004). It has been hypothesized that twins may have a favorable social environment due to interactions with, and social support of the co-twin (Pulkkinen *et al.*, 2003). In order to test whether the findings of this thesis are generalizable to non-twin children, it is important to compare the results on aggression regulation following social evaluation with a sample of non-twins. Recently, several other research facilities have started to use the Social Network Aggression Task, and combining these samples will enable such direct comparisons.

Multiple samples *vs.* Massive samples

A twin study provides the additional possibility to test a specific psychological construct in two similar samples (one co-twin in each), thereby replicating findings within a study. Replication designs are very useful for testing the robustness and reproducibility of results (Schmidt, 2009; Open Science, 2015). Examples of multiple samples within one study are provided in this thesis in chapter 2 (pilot- test- replication design), chapter 5 (ROI selection in independent sample) and chapter 7 (functional connectivity in two independent samples). The findings of thesis also showed that high statistical power is needed to detect

subtle brain-behavior associations, specifically in children. That is, using multiple smaller samples (n<30) in chapter 2 did not reveal the social rejection specific neural activity that was found using a larger sample (n>300) in chapter 4. Moreover, the independent sample for ROI selection (n=41) in chapter 5 had too little statistical power to reveal the whole brain-behavior associations that were reported with the exploratory analyses (n>300). An important methodological objective that follows from this thesis is that multiple samples are not necessarily better than large samples (or vice versa), but that they serve different purposes. Replicability is extremely important for confirming findings (Ioannidis, 2005; Schmidt, 2009), but for explorative discoveries we need a lot of statistical power and therefore large samples (Mumford and Nichols, 2008; Button *et al.*, 2013). This is especially true for developmental neuroscientific studies, as the attrition rate in children often is high (O'Shaughnessy *et al.*, 2008; Raschle *et al.*, 2012; Fassbender *et al.*, 2017a).

Control your head motion: Attrition biases

Children are more prone to head motion during the MRI scan than adolescents and adults (O'Shaughnessy *et al.*, 2008; Raschle *et al.*, 2012; Fassbender *et al.*, 2017a). To limit the confounding effect of head motion on MRI findings, it is important to exclude participants that exceed a specific threshold of head motion (Power *et al.*, 2015). This often results in an underrepresentation of children in cohort-sequential longitudinal studies, an issue that can be overcome by oversampling children during data acquisition. However, excluding participants who display excessive head motion might induce an additional bias: it is likely that participants who have difficulty regulating their head motion also experience difficulty regulating their emotions and behaviors. Indeed, studies showed a significant association between head motion and motor control (Zeng *et al.*, 2014; Ekhtiari *et al.*, 2019). This indicates that participants with the most behavioral control problems are the first to be excluded in MRI research (Kong *et al.*, 2014). This bias is almost insurmountable, but must be kept in mind when interpreting neuroscientific studies on emotion regulation and behavioral control. More and more methods to deal with head motion during MRI scan acquisition are being developed, for example by using real-time monitoring of head motion (Dosenbach *et al.*, 2017) or customized head molds (Power *et al.*, 2019), which might enable future studies to exclude less participants and thereby minimize attrition bias.

fMRI: State of mind or state of mess?

The reliability of functional MRI, specifically experimental (task-based) fMRI has been heavily debated in recent years (Nord *et al.*, 2017; Elliott *et al.*, 2019b; Frohner *et al.*, 2019). The variability observed in fMRI blood oxygen level dependent (BOLD) signal and the poor test-retest reliability in developing populations is a big concern for the field of developmental neuroscience (Herting

et al., 2018). Test-retest reliability is the extent to which a measure produces stable outcomes across different time points under comparable conditions (Dubois and Adolphs, 2016). Prior longitudinal developmental studies, including chapter 5 of this thesis, reported low intra-subject stability across different scan session (for an overview see Herting *et al.* (2018)). These could either reflect individual variability over time or might reflect unaccounted-for noise in the fMRI measurement (Dubois and Adolphs, 2016). The behavioral genetic analyses on fMRI in chapter 4, 5 and 6 showed that a large proportion of variance was explained by the E-factor, which includes both unique environmental influences and measurement error. An important objective for future research is to disentangle between the influence of unique environment and measurement error, for example by accounting for intra-subject fluctuations using repeated measures (Ge *et al.*, 2017). Using such a repeated measures approach, one can tease apart the stable effects (which are due to unique environment) from the transient effects (which might arise from measurement error) (Ge *et al.*, 2017).

Heritability estimates for fMRI are often lower than for structural MRI (sMRI) (Jansen *et al.*, 2015). Similar to the difference between questionnaire data and experimental data, sMRI can be seen as a trait-like measure of the brain, whereas fMRI provides a state-like measure (Greene *et al.*, 2018a). Indeed, questionnaire data often shows higher heritability and test-retest stability than experimental studies (Tuvblad and Baker, 2011), that are aimed to induce a specific state. A state can be defined as *"the particular condition that someone is in at a specific time",* and by this definition it seems reasonable that there is more intra-individual variability across time for experimental (fMRI) studies. An important benefit of the state-inducing ability of fMRI is that it can isolate specific aspects of complex behaviors. A broad range of literature - including chapter 3, 4 and 5 of this thesis- have shown that experimental fMRI is meaningful in relation to behavior and can provide valuable information about the underlying mechanisms of specific behaviors. It should be noted that the field of developmental neuroscience, and specifically the use of longitudinal experimental fMRI studies, is still young (Crone and Elzinga, 2015; Herting *et al.*, 2018). Perhaps the strength of fMRI lies in the combination of different MRI modalities (Dubois and Adolphs, 2016). That is, experimental fMRI might be used to detect meaningful associations between behavior and brain regions, which can be further examined by studying the stability or heritability within this region using additional MRI metrics (Greene *et al.*, 2018a; Elliott *et al.*, 2019a). This would provide an in-depth examination of both trait-like and state-dependent features of brain-behavior relations.

Future directions

Based on the main scientific outcomes of this thesis, and taking into account the methodological considerations that arose from the different studies, I have formulated three objectives that are important for future research.

Combined forces: Multimodal brain imaging

In order to use experimental neuroimaging to its full potential, while taking into account the limitations that it entails, it is important to combine different MRI metrics. Aggressive behavior and emotion regulation have been studied using different MRI methodologies, such as structural anatomy (Bos *et al.*, 2018), experimental fMRI (Ochsner *et al.*, 2012), functional connectivity (Fulwiler *et al.*, 2012) and structural connectivity (Olson *et al.*, 2009; Peper *et al.*, 2015), but the number of studies that combined different metrics is limited. Nevertheless, most theoretical frameworks suggest that behaviors and emotions are regulated through communication between specific brain regions that are part of a large and complex brain network (Casey, 2015). To empirically examine the complex features of the developing brain and its association with behavioral outcomes, a multimodal brain imaging approach is needed.

Individual differences in developmental trajectories

The single time-point studies in this thesis (chapters 2, 3, 4, 6, 7) provide starting points for understanding social emotion regulation in the childhood brain. To understand the developmental trajectories of social emotion regulation, however, we need longitudinal studies (Crone and Elzinga, 2015; Telzer *et al.*, 2018). Although I made a start with this approach in chapter 5 and 8, it should be noted that two measures are only slightly better than one. Three or more measures are needed to capture complex developmental trajectories, as this allows investigating both linear and non-linear individual growth trajectories (Madhyastha *et al.*, 2018). Both behavioral outcomes (such as reward sensitivity or emotional reactivity) and brain development have shown non-linear development across childhood, adolescence and adulthood (Galvan, 2010; Silvers *et al.*, 2012; Wierenga *et al.*, 2018a). Most of these studies had an underrepresentation of children, resulting in more uncertainty (larger confidence intervals) in developmental trajectories across childhood. The L-CID sample consists of a unique twin sample that will be followed for a total of six years (Euser *et al.*, 2016), including three MRI measures. This will allow for examination of individual differences in developmental trajectories across childhood and emerging adolescence. Additionally, due to the large sample size and therefore excellent statistical power, we can examine how childhood brain development

can predict adolescent behavior and further explore childhood as a window of opportunity.

Social communication of digital natives

Today's children are the first generation to grow up with unlimited internet access, enabling to be constantly connected to a complex and intense (digital) social network. Despite the fact that social media is everywhere around us and used by almost everyone on a daily basis, very little scientific research has been conducted on the effects of social media on the developing brain (Crone and Konijn, 2018). The studies in this thesis provide a starting point by unraveling the neural mechanisms of social evaluation in childhood. An important question for future research is whether individual differences in sensitivity to social evaluation are related to individual differences in real-life (digital) social interactions. Numerous studies have used real-life social media monitoring (for example see Montag *et al.* (2014)), mostly in combination with questionnaire data. Although this can provide insight on behavioral correlates, the covert neural mechanisms involved in social media remain unknown. The novel approach of bringing together both real-life social media monitoring, as well as innovative developmental neuroimaging will result in cutting edge research and can provide insights through a neuro-mechanistic approach.

Conclusion

This thesis provides a comprehensive overview of the underlying mechanisms of social emotion regulation in childhood. The studies show that our brain is prone to signal for socially relevant information, irrespective of its valence. This network of social saliency is already present in childhood, indicating this might be a core social mechanism. The thesis additionally shows that social rejection is often followed by behavioral aggression, and regulation of these retaliation emotions is related to control mechanisms of the DLPFC. The results are in line with previous neurodevelopmental models, which highlight the importance of top-down control of prefrontal regions over bottom-up processing subcortical-affective regions. As complement to these models, the results show that the vast architecture of functional subcortical-PFC brain connectivity is already in place in middle childhood and suggest fine tuning of (social evaluation) brain networks across childhood, highlighting the need to incorporate childhood into developmental models of social emotion regulation. Neuroimaging research, specifically neuroimaging in children is prone to challenges and several methodological considerations need to be taken into account when studying the childhood brain. In spite of these difficulties, studying childhood brain development has the potential to provide important insights into a unique developmental window of opportunity.

Table 1. Meta-analytical activation for social salience, social rejection and social acceptance. Results are based on 3 studies using the Social Network Aggression Task (chapter 3, 4, and 5). Note that there was no significant activation reported for the social rejection contrast in chapter 3. Meta-analytical results were obtained with GingerALE, using p<.001 and volume > 300 mm^2.

Anatomical Region	x	y	z	ALE	Z	p
Social Salience (positive and negative > neutral social feedback)						
Insula (left)	-32	26	-6	0.02	5.54	<.001
Insula (left)	-30	12	-16	0.02	4.83	<.001
Insula (left)	-44	16	-4	0.02	4.74	<.001
Insula (left)	-38	22	-16	0.01	3.51	<.001
Insula (left)	-30	18	0	0.01	3.17	0.001
Insula (right)	36	24	-12	0.02	4.74	<.001
Insula (right)	38	30	4	0.01	4.18	<.001
Medial frontal gyrus (right)	12	48	13	0.01	3.62	<.001
ACC gyrus	0	46	10	0.02	5.11	<.001
ACC gyrus	0	38	16	0.01	3.36	<.001
ACC gyrus	2	56	12	0.01	3.17	0.001
Occipital lobe (left)	-48	-76	-2	0.02	5.85	<.001
Occipital lobe (right)	48	-72	-4	0.02	5.03	<.001
Occipital lobe (right)	50	-62	-2	0.01	3.35	<.001
Occipital lobe (right)	50	-78	6	0.01	3.1	0.001
Social Rejection (negative > positive social feedback)						
IFG (right)	57	32	4		4.48	<.001
IFG (left)	-45	26	-8		5.69	<.001
IFG (left)	-52	28	4		4.22	<.001
Insula (left)	-38	-16	26		4.22	<.001
MPFC	-12	60	25		4.32	<.001
MPFC	-6	54	30		4.05	<.001
Cuneus (left)	-8	-97	12		4.83	<.001
Cuneus (right)	26	-91	16		5.11	<.001
Social Acceptance (positive > negative social feedback)						
DLPFC (right)	39	34	40		4.19	<.001
SMA (right)	26	6	56		4.4	<.001
Culum of cerebellum (right)	4	-74	-2		5.23	<.001
Occipital lobe (left)	-18	-85	-6		4.34	<.001